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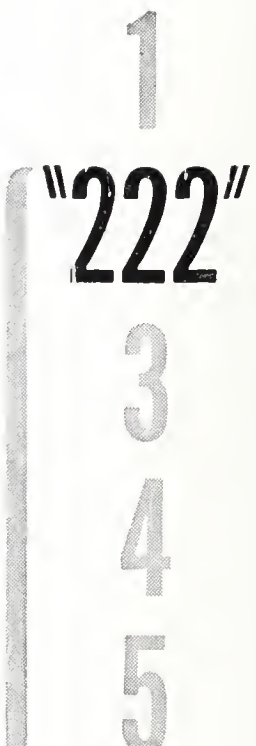

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Editorials

The editor considers himself fortunate that a distinguished scientist and humanitarian, Linus Pauling, has written the leading article for this issue. The *curriculum vitae* on Dr. Pauling was released for the Journal's use by the Center for the Study of Democratic Institutions. This organization was established to promote the principles of individual liberty expressed in the Declaration of Independence and in the Constitution of the United States of America; towards these ends Dr. Pauling has directed his life's work.

For some of our graduating students, their future work will be in the reserve of biological laboratories. Perhaps here there will be interest in the problems mentioned by Dr. Pauling. The suggestion that the physical chemical properties of liquid water and its solutions have to be fully understood before there is a complete understanding of the nature of physiological processes, is of considerable interest. This can be further extended if one considers that the distribution of soluble constituents between dispersed phases in complicated colloidal systems critically determines the stability and many physical properties of these systems.

For the majority of the graduating students their work will be more directly connected with the immediate needs of society in their role of physician. For a few it will not be enough to battle human disease but it will be necessary to worry about economic, political and social injustices. Perhaps they will understand the purposes of men like Dr. Pauling by shared sympathies, and in so doing will succeed in freeing the world community from sufferings caused by war, hunger, disease and illiteracy.

The paper by Dr. Linda Geiger was written as one requirement for the degree of B.Sc. (Medicine). This demands that three consecutive summers, of three months each, be spent in study and research under the sponsorship and guidance of a staff member designated by the head of department in which the candidate elects to work. Dr. D. W. Clarke was the staff advisor for Dr. Geiger. It is of added interest that Dr. Clarke received part of his graduate training under Dr. Pauling at the California Institute of Technology. At that time Dr. Pauling was interested in serological reactions and the structure of antibodies.

The third paper in this Journal is a précis of a 25,000-word essay which was nominated for the Charles E. Frosst Scholarship in 1964. This original paper represented a prodigious amount of work by the author and demanded not only an understanding of the basic physiological processes but an exhaustive search of the literature. While the 4000-word paper in this Journal is, by necessity, shortened considerably, it serves to illustrate the quality of work which is necessary to win this honour. Indeed, the work will excite some readers to possible avenues of research; whether it be in an expansion of the understanding of the molecular basis of drug action or in more lucid appreciation of the mechanism of polymerization of the fibrinopeptides to the insoluble cross-linked fibrin molecules. In this end consideration of the theories of Florey, Staudinger, Carruthers and many others will possibly be utilized by the Medical profession to explain the physical chemical properties of this polymeric system.

The paper by E. M. Sellers represents a further deviation from the content of previous journals in that it discusses a field research problem. It is perhaps the most difficult type of research problem for the student to attempt to answer. Rather than discussing all the data obtained and thus reach certain conclusions biased by limited time and subjective influences, the author has decided to give only a general impression of the problem in this issue.

Previous papers by students describing their summer research problems have generally suffered little editorial criticism because of the editor's lack of familiarity with the various areas of research. In an effort to alter the situation such that both the contributors and readers could benefit from the Research Issue, it was decided to insist that the student's staff advisors be given co-authorship where this was applicable. Towards this end, Dr. Cowan and Dr. Rewcastle have been most helpful in accepting this responsibility with their respective advisees, M. Baker and N. Iscove.

E.G.B.

EDITORIALS

That one of the issues of the University of Toronto Medical Journal should be devoted to student research is fast becoming a tradition, and one with which the Editors take no exception. This year the issue has been enhanced by a number of factors. The Associate Editor is himself engaged in work in the area of physical-chemical properties of protein solutions, and his close contact with active research makes him an able critic of the submitted papers. An innovation introduced by the Associate Editor was a collaboration between the student and his research advisor, where possible, in the preparation of the paper. This has resulted in some very fine papers, as well as a more thorough understanding of the topic, and is a feature to be encouraged in future issues.

This third Student Research issue again presents a unique opportunity for the undergraduate to write concerning a subject of which he has both theoretical *and practical* knowledge, and for the reader to appreciate the comprehensive understanding that can be gained in a summer of research into some aspect of medicine. Encouragingly, more and more frequently the medical student is able to pursue such knowledge, through the interest of researchers in having undergraduates spend their summer months with them, and through the availability of funds to supplement these same researchers' often times rather meager research grants.

Both the future clinician and reseacher will value such a research experience. For the individual hoping to become involved in full time research the positive factors are obvious. A more acute evaluation and an appreciation of the many pitfalls of the experimental situation will broaden the background of the full time clinician. Perhaps most important, those who plan to partition their time between clinical work and research, whether the latter be in the clinical or basic science area, will be aware of the time must be allotted, and the high criteria to which one must adhere, if competent research is to be accomplished. While the student may be disappointed with the slowness at which his work progresses and frustrated by the many practical difficulties which he encounters, he will, nevertheless, gain satisfaction from the insight which he achieves into this most important field of medicine.

B.E.K.

With this issue, volume XLII of the University of Toronto Medical Journal comes to press.

For the third time in five years a new cover design is introduced. But this cover is unique, for unlike previous years, it has not been the creation of the Editorial staff. On the contrary, it is the work of a professional graphic artist. The Editors are grateful to Mr. Ralph Bongard for a design, which we hope will become a permanent and aesthetic segment of the Journal. We think that he has already succeeded.

This issue also marks the advent of a new printer to the Journal. The memories of the tardy publishing dates of volume XLI are all too fresh. Therefore we hope (and trust) that *Maple Leaf Press* will aid in returning the Journal to its traditional virtues of punctuality and accuracy.

Last, volume XLII commences with Student Research and ceases with The Lung. In the interim there will be issues on Malignancy, Virology, Immunity & Hypersensitivity, and Sex. We think that the subject matters are all chimeras — mixtures of the broad and the specific, the basic and the applied, the research and the clinical, imbedded in the tissues of medical science. Most of all, we feel that they are interesting and exciting and we think you will too.

J.S.B.

Linus Pauling was a member of the staff of the California Institute of Technology for more than forty years, beginning in 1922. In 1963 he joined the staff of the Center for the Study of Democratic Institutions, with an appointment that gives him freedom, as he states, to divide his efforts among the fields of principal interest to him—science, medicine, and world peace.

Dr. Pauling's early researches, many of which were carried out in collaboration with colleagues and students, were centered about the problem of the structure of molecules and crystals and the nature of the chemical bond. For this work he was awarded the Nobel Prize for Chemistry for the year 1954. In 1934 he and his co-workers began a series of experimental and theoretical researches in molecular biology and medicine, which in the course of time encompassed the magnetic properties of hemoglobin, the three-dimensional structure of protein molecules, the structure of antibodies and the nature of serological reactions, the discovery of the abnormal human hemoglobins that are related to certain diseases, the bearing of protein structure on the evolution of species, and the development of a molecular theory of general anesthesia.

At the end of World War II he was awarded the Presidential Medal for Merit for his work on rocket propellants and other explosives, on an oxygen deficiency indicator for submarines and aircraft, and on the development of a substitute for human serum in medical treatment, "among other contributions to the war effort."

Dr. Pauling's work to abolish war began in 1945, immediately after the use of atomic bombs at Hiroshima and Nagasaki, with a series of public lectures about the nature of nuclear weapons and nuclear war. It was intensified in 1946, when he became associated with Albert Einstein and seven other scientists in the Emergency Committee of Atomic Scientists. During recent years his work for world peace has for the most part been carried out in collaboration with his wife, Ava Helen Pauling. Together they have given lectures about peace in most of the states of the United States and in twenty-five other countries.

Hans Bethe, distinguished physicist of Cornell University and former member of the President's Science Advisory Committee, has said: "It should be realized that the limited test-ban . . . probably would not exist if it were not for the scientists . . . Pauling . . . got the Nobel Prize for his role in this—I think rightly . . . Pauling certainly had great influence on people both in this country and abroad. Without his awakening of the public conscience on this issue there would not have been any pressure on governments, and there would not have been any test-ban."

In addition to scientific articles and books, Dr. Pauling has published about one hundred articles on world peace and related subjects, and one book on the subject, entitled No More War!

The Possibilities for Further Progress in Medicine through Research

Linus Pauling, Ph.D.

When I was born, in 1901, the electron had been discovered, but the atomic nucleus had not. In 1912 Rutherford reported his experiments on the scattering of alpha particles, and his interpretation of them as showing that the mass of an atom is for the most part concentrated into a very small particle, carrying a positive electric charge—the nucleus of the atom, about which the electrons move. Most chemists were confident that the structural formulas that they wrote for chemical substances bore a close relation to the actual arrangement of the atoms in the molecule, relative to one another, but there was little expectation that in a decade or two it would become possible to determine the structures of molecules and crystals with great accuracy.

The first measurements of interatomic distances were made by W. H. and W. L. Bragg, by application of their method of determining the structure of crystals by X-ray diffraction. This method was soon supplemented by others: the analysis of band spectra, the electron-diffraction technique for gas molecules, and so on. By 1940 precise structural information had been gathered for hundreds of gas molecules and hundreds of crystals.

It then became possible to ask significant questions about the molecular structure of the human body and the molecular basis of disease and of therapeutic treatments. A few of these questions have been answered. We have begun to talk about molecular biology and molecular medicine, and to have the feeling that biology and medicine are just at the beginning of a period of extraordinary development, a Golden Age similar to that through which physics and chemistry have passed during the last seventy years.

Even though powerful methods of experimental investigation have been developed during recent decades, we must recognize that the problem of determining the molecular structure of the human body is an extremely difficult one, and that progress may be slow. I may illustrate this point by mentioning the substance that constitutes a larger fraction of the human body than any other substance—the substance water. We know a great deal about the isolated water molecule; we know that the average distance between the center of the oxygen atom and the center of each of the two hydrogen atoms is 0.965 Å, and that the H-O-H bond angle has the value 104° 31′. Also, we know the detailed structure of ice. But the water molecules in the human body are not present as isolated water molecules or as ice; they are present as liquid water, containing various substances in solution. We are still largely ignorant about the structure of liquid water and the structure of aqueous solutions in general. We shall continue to have difficulty in understanding the nature of physiological processes so long as we have not solved the problem of the nature of liquid water and of its solutions.

A most striking development of recent years has been the increase in our knowledge about the structure of proteins and of nucleic acids. A number of diseases are now known to result from a gene mutation that leads to the substitution of a single amino-acid residue for another in a polypeptide chain of some protein in the human body. For example, in the disease sickle-cell anemia it is known that the two beta chains of the hemoglobin molecule (rather than the two alpha chains) carry the abnormality, that the abnormality is in the sixth position from the free amino end of the beta chain (which contains 146 amino-acid residues), and that it involves the introduction in this position of a valine residue, in place of the glutamate residue that is found there in normal adult human hemoglobin. Moreover, rapid progress is being made in the attack on the problem of the nature of the genetic code, and it is likely that within a year or two it will be possible to say just what substitution of one nucleotide for another in the polynucleotide constituting the gene for the synthesis of the beta chain of hemoglobin has taken place during the mutation from normal to sickle-cell hemoglobin.

We can be confident that in one or two decades similar information will become available for hundreds of diseases, and that this information will in the course of time lead to improved methods of prevention and treatment.

There is, moreover, the possibility of a great improvement along another line—the understanding and control of mental disease. I estimate that there is a strong hereditary factor in 80% of the cases of mental disease. There is, of course, also in many cases a significant environmental factor. A human being may sometimes have a hereditary nature such that he can withstand great environmental stress, whereas for other human beings their hereditary character is such that even the minimum amount of environmental stress is enough to cause serious mental disease. About half the hospital beds in the United States are occupied by mental patients. About 10% of the American people spend some time during their lives in a mental hospital. I believe that through research on the molecular basis of mental disease it will be possible to decrease its incidence significantly.

One way to attack the problem of mental disease is to carry on research on the structure and function of the brain. I believe that it will be possible, perhaps within twenty years, to determine the nature of the electric oscillations in the brain that constitute consciousness and ephemeral memory, the nature of the material pattern in the brain that constitutes permanent memory, and the nature of the interactions between the electric oscillations and the material pattern. I believe that it will be possible to discover the important molecules involved in the process of thinking, to determine their structure, and in the course of time to discover what the molecular abnormalities are that lead to mental deficiency and to mental illness. Molecular biology and molecular medicine are new fields of science that can be greatly developed for the benefit of mankind.

The young man or woman who is now attending medical school and preparing to be a physician is faced with a problem that will be with him throughout his life. The nature of medical practice is going to change, more and more, during the next few decades, as the result of discoveries that are made about the human body, the vectors of disease, and the molecular basis of drug action. It will be important that the physician understands these changes that are brought about by the new discoveries. To prepare himself to follow the progress of medical research in the future, the student of medicine can do nothing better than to participate in research himself. I am accordingly pleased that I can be associated with some of the University of Toronto medical students who have been carrying on research and are publishing accounts of their work in the Research Issue of the University of Toronto Medical Journal.

The Effect of EAE Serum on the Metabolism of Rat Brain Slices[+]

Linda Geiger, M.D. 6T4*
D. W. Clarke, Ph.D.†

INTRODUCTION

Demyelinating diseases have remained a source of medical perplexity for more than a century. In the 1830's, Curveilhuir and Carswell first described multiple sclerosis as a clinical and pathological entity (1). Subsequently, there were reports of neurologic symptoms differing from rabies in patients vaccinated with Pasteur's antirabitic vaccine which was introduced in 1885(2). Several investigators linked this post-vaccinal reaction to the effects of injection of normal nervous tissue in animals (3). In a more comprehensive study, Rivers and Schwentker, in 1935, produced a consistent clinical picture in monkeys by multiple injections of emulsions of brain and spinal cord. They described the histology and found it surprisingly comparable to the human demyelinating diseases which were being discovered, described and classified at this time (4).

The addition of Freund's adjuvant to the spinal cord homogenate greatly enhanced the incidence and severity of the laboratory disease, Experimental Allergic Encephalomyelitis (EAE), so that it now became a useful tool to view the demyelinative process (5,6). Many characteristics of EAE seemed to parallel immunologic reactions; therefore, the search for supportive evidence for an immune etiologic basis was begun. As investigative procedures developed, their adaptability to the study of human demyelinative conditions was considered in terms of diagnostic tests as well as etiologic inquiry.

The aim of this present research was to explore the possibility of studying demyelinative processes "in vitro". It was postulated that if EAE serum contained an "active factor", there would be measureable metabolic changes in brain tissue exposed to this serum. Two metabolic parameters, the glucose uptake and the fatty acid output, were chosen as the subject matter. Rat cerebral slices provided the metabolizing tissue.

REVIEW OF THE LITERATURE

1. *Myelin*

Myelin is a lipoid material which forms a sheath around certain nerve fibres. In the peripheral nervous system the sheaths are encased by Schwann cells. The continuity of the sheath is regularly interrupted at points called the nodes of Ranvier. At the nodes the neurolemmae, or Schwann cells, dip down to touch the axon. One Schwann cell spans the sheath between nodes. Electron microscopy and special staining techniques have shown myelin to have a regular concentric lamellar structure with each lamella divided into five layers (7). The plasma cell membrane of the Schwann cell is continuous with these layers. It has been proposed that the spiraling invagination of the Schwann cell plasma membrane is responsible for the layered structure of the myelin. This structure becomes evident at the nodes since the innermost turn of the membrane has the smallest width. As spiraling continues, the Schwann cell growth also takes place so that layers become progressively wider.

There are no Schwann cells in the central nervous system where oligodendrocytes, and possibly astrocytes, may play a comparable role. However, their association with the myelin sheath is not so intimate a one as that of the Schwann cell. Glial processes form flat sheaths which then surround the axons. Growth occurs at both advancing tips unlike the Schwann cell membrane where it is probably limited to a single invaginating tip. In this manner, several axons may be enveloped by one glial process (7).

2. *Myelination*

In the human foetus myelination begins around the fourth gestational month. The process proceeds from the cell body towards the fibre termination. The sensory tracts are myelinated first, followed by those of the motor pathways. These are not completely myelinated until two years of age and, probably, the total process continues until twelve or fourteen years of life (8).

3. *Neurochemistry*

Brain tissue has a high lipid content, 64.4% of the dry weight of tissue in the white matter and 34.2% in the grey matter. The water content averages about 40% and cholesterol levels are high. It is now possible to study the composition of white matter in more detail. Solvent extraction procedures and chromatographic fractionation techniques have been developed making a standard breakdown process feasible (9). The result is a long list of components which are described briefly below.

A. Proteins

The two main protein fractions isolated are proteolipids and neurokeratin.

[+]Sponsored by the Multiple Sclerosis Society of Canada.

*Junior Interne, Toronto Western Hospital, Toronto, Ontario. This paper is taken from Dr. Geiger's B.Sc. (Medicine) Thesis, 1964.

†Professor, Banting and Best Department of Medical Research, University of Toronto.

i) *Proteolipids:* These are abundant in white matter, constituting 2% of the fresh tissue weight. They can be subdivided into three types A, B and C containing 15-25%, 50% and 75% protein respectively. These types are differentiated by their precipitating characteristics in chloroform-methanol solution.

ii) *Neurokeratin:* This is a proteolytic enzyme-resistant residue of myelin which may be simply an artefact of a drastic extraction process. More recently, gentler techniques have yielded trypsin-resistant-protein-residue (TRPR) with properties close to those of neurokeratin. Neurokeratin results when TRPR is subjected to the techniques of extraction which give neurokeratin from the raw materials (10).

B. Lipids:

The lipids in myelin may be classified as follows (11):

(i) Sterols, e.g. cholesterol

(ii) Phosphatides: these are compounds which contain phosphate esters

- (a) phosphoglycerides—lecithin
  —phosphatidyl ethanolamine
  —plasmalogens (which have an aldehydrogenic residue)
- (b) phosphoinositide
- (c) phosphosphingosides—sphingomyelin

(iii) Sphingosides: these are sphingosine-containing lipids

- (a) cerebrosides—(N acyl sphingosyl galactosides)
- (b) sulfatides—(monosulfate esters of cerebrosides)
- (c) mucolipids—e.g. sialic acid (acetyl neuraminic acid)

plus gangliosides
strandin

Chemical Changes during Myelination

The chemical composition of white matter is not constant but varies during the phases of its development: preceding myelination, during active myelination and after myelination is completed. It is found that as age increases, water content decreases. Total hexosamine and cholesterol ester levels, which are high in early development, fall to trace levels in the post-myelination stage (11). Cerebrosides and proteolipids are at very low levels in the newborn mouse and increase rapidly as myelin appears. During myelination, chromatographic studies show an increase in plasmalogen and sphingomyelin phosphorus while lecithin phosphorus decreases. Incidentally, a comparison of the myelinated and unmyelinated fibres of the peripheral nervous system demonstrates that a major difference of myelination lies in a higher ganglioside content (10).

The development of myelin components and the metabolic activity of myelin during the various stages mentioned has been studied by "in vivo" tracer experiments using such compounds as C^{14} glucose, C^{14} acetate, C^{14} labelled fatty acids, etc. (12, 13, 14, 15).

4. *Structure of Myelin*

Analysis of all the chemical information plus the results from X-ray crystallography and polarization microscopy studies on myelin prompted Finean (7) to propose a molecular structure of the myelin sheath.

5. *Demyelination*

A. Pathology

Loss of the myelin sheath is a common response of the white matter to many different pathologic stimuli. Classically, it is described by the sequelae following the severance of the nerve (Wallerian degeneration); electron microscopic studies have shown the following sequence of events (7):

(i) Forty-eight hours after injury the axon is completely degenerated;

(ii) Within ninety-six hours the myelin sheath shows lamellar splitting and the Schwann cell is hypertrophied;

(iii) In one week, masses of fragmented disorganized myelin are visible in Schwann cell cytoplasm.

The pathochemical changes involve a decrease in phospholipids and cholesterol content with a rise in esterified cholesterol. Hexosamines may also be elevated (16).

Disintegration, as described above, must be distinguished from primary demyelinative processes in which the prominent feature is demyelination *per se*. Adams proposed the following histologic criteria for primary demyelinative disorders (17):

(i) Destruction of the myelin sheath with relative sparing of the other elements, especially the axons and cell bodies;

(ii) Disseminated foci which are perivenous in distribution.

Several pathologic entities fulfill Adams' criteria to a greater or lesser degree; multiple sclerosis is the commonest but others, such as post vaccinal encephalomyelitis, acute necrotizing leuco-encephalomyelitis, Schilder's disease et al, are included.

B. Biochemical Considerations

On the basis of biochemical findings, the primary demyelinative disorders have been divided into three categories (16).

(i) *Myelinoclastic:* Multiple sclerosis is the main condition under this heading. Early changes show a decrease in total phospholipids, tissue weight and an increase in esterified cholesterol and neuraminic acid. Chromatography suggests that there is a disproportionate loss of plasmalogen.

(ii) *Reactive and Inflammatory:* Schilder's disease is included here (it has a more violent pathologic reaction). The losses in phospholipid and total cholesterol are much greater than in multiple sclerosis. However, there is little rise in esterified cholesterol and a moderate rise in hexosamine (residual fraction only).

(iii) *Dysmyelinative:* The conditions included in this category are disorders with abnormal or defective myelin anabolism such as enzymatic defects in the production of cerebrosides and sulfatides. A representative disorder is metachromatic leukodystrophy. Here the general decreases previously described are present plus great increases in the total hexosamine (lipid, water and residual fractions). There is elevation of sulfatides.

The lipoidoses are omitted from this categorization since, although demyelination is often present, it generally does not constitute the major problem in these conditions.

EXPERIMENTAL ALLERGIC ENCEPHALITIS

Histologic studies first related EAE to the primary demyelinative disorders (18, 19, 20). Like multiple sclerosis, the gross findings are usually unremarkable but, on section, small petechial lesions are seen. These are present in the brain stem, spinal cord, optic nerves, cerebral and cerebellar white matter. Microscopically, the lesions are related to blood vessels. Very early, the cellular infiltration is polymorphonuclear in nature with occasional eosinophilia and perivascular haemorrhage. Myelin degeneration is present with relatively good axonal preservation. Later the lymphocytic and monocytic response becomes more prominent with microglial proliferation as well. Sudanophilic products of degeneration appear. The similarity of the laboratory disease to the human picture is striking enough to warrant comparison.

As yet there have been no studies reported on the neurochemical changes in brain and spinal cord in EAE.

A. Immunology:

EAE fits very neatly into that category of disease states which result from antigen-antibody interaction. A few pertinent immunologic principles are reviewed before the characteristics of EAE, which form the present evidence for its immune etiology, are described (21).

Types of Reactions

1. *Immediate:* The typical example is the phenomenon of anaphylaxis. This type of reaction is characterized by an immediate response with demonstrable circulating antibodies, the ability to transfer the disease using serum and the presence of target organs.
2. *Delayed:* The prototype is the tuberculin reaction. In sensitized individuals there is a slowly evolving inflammatory response. Homograft rejection is of this type. The characteristics of delayed sensitivity include:
   (i) the absence of demonstrable humoral antibodies
   (ii) the inability to transfer disease by serum but by sensitized cells such as lymphocytes
   (iii) the lack of a specific reactive tissue
   (iv) a time delay between contact and the expression of reaction
   (v) the specificity of the cytologic event. The lesion is generally mild. It is associated with blood vessels, especially venules. The infiltrate consists of mononuclear cells.

Lately it has been theorized that basically there is no difference between these immunologic mechanisms. The distinctiveness of the reactions is due to differences in antigen-antibody affinity. Manipulation of the antigen antibody ratio has enabled reversals in reaction type to be produced (22).

B. Immunologic Basis of EAE

1. *Nature of Disease:*

   Like other delayed hypersensitive states EAE requires a latent period prior to the development of the disease. The lesions produced are perivenous with a prominent mononuclear response.

2. *Antigen:*

   Many efforts have been made to isolate the active encephalogenic agent from CNS tissue. It has been shown that myelin-rich fractions are more active than fractions derived from grey matter (23). Thompson et al recently described a fraction containing a phospholipid-protein complex which they claim contains the total encephalogenic activity of the white matter (24). It is also interesting to note that Kabat et al produced EAE in monkeys using autologous brain tissue obtained by lobectomy (25). Freund's adjuvant, as mentioned before, greatly enhanced the immunological response.

3. *Route of Injection:*

   The most effective route of injection for EAE production is the intradermal route or foot pad (3).

4. *Immunologic Tolerance:*

   Suppression of disease has been produced by pretreating immature animals with antigen (26). Small repeated doses of spinal cord minus adjuvant given by subcutaneous route have been reported to be protective in the adult animal (27).

5. *Transfer:*

   No transfer has been accomplished using blood serum or cells (28). However, Paterson claims to have transferred the disease by means of sensitized lymph node cells (29); these results have not been confirmed. In rats, parabiosis has effected the transfer of disease (30).

6. *Prevention and Suppression:*

   (i) *Irradiation:* Total body radiation increases the production of EAE (31). However, if irradiation is limited to the regional lymph node then suppression is achieved (2).

   (ii) *ACTH and Cortisone:* These hormones are effective in decreasing the incidence of the disease as well as its severity (32, 33).

   (iii) *6-Mercaptopurine:* If this drug is administered prior to challenging the animal with the antigen, the incidence of the disease is diminished (34).

7. *Evidence of Antibody Activity:*

(i) *Complement Fixation:* An antibody causing complement fixation is present in EAE serum but its presence cannot be correlated with the disease findings. Therefore, it cannot be implicated as operative in an etiologic mechanism (35).

(ii) *Coon's Fluorescent Technique:* Rose et al treated normal cord slices with serum from guinea pigs with EAE. Subsequently, these slices were treated with an appropriate fluorescent anti-gamma-globulin. Fluoromicroscopy illustrated antibody fixation at sites which correlated well with the known positions of the myelin sheaths (36, 37).

(iii) *"In Vitro" Demyelination:* Bornstein and Appel developed tissue cultures of myelinated new-born rat cerebellum. When these cultures were well established they were treated with EAE and normal serum and followed by a special high-powered microscopic set-up. Demyelinative changes were noted as early as one hour and complete in twenty-four to forty-eight hours. These changes were only seen in the presence of EAE serum; neither normal nor control sera caused demyelination. In some cases, the removal of the EAE serum allowed remyelination. Whether or not the demyelinative agent is an antibody cannot be stated but, this explanation seems plausible (38, 39).

METHODS AND MATERIALS

1. *EAE Production*

The spinal cord was removed from normal adult guinea pigs and the meninges and blood vessels cleared as much as possible. The cord was then homogenized in Freund's adjuvant (0.5 gm to 3 cc). The homogenate was injected intradermally in 3 or 4 sites in the back of 250-600 gm. mature guinea pigs (irrespective of sex). Sufficient homogenate was injected to raise a bleb about 0.5 cm. in diameter. An alternative route of injection used was the hind foot pad.

After a period of time varying from eleven to twenty-eight days, the animals developed weight loss, weakness and paralysis which was most pronounced in the hind limbs, convulsions, urinary retention and bowel dysfunction. When these signs were fully developed, the blood sample was collected.

2. *Preparation of Serum*

The experimental and control animals were anaesthetized with sodium pentothal (0.5 cc/kilo body wt.) and ether. The abdominal cavity was opened and the inferior vena cava punctured with a No. 21 needle. About 12-20 cc was thus collected in a heparinized syringe. The blood was centrifuged and the serum separated. This was then frozen and stored at below 0°C.

Some of the serum was treated with equal portions o saturated ammonium sulphate by dialysis over a 2-hou period. The mixture was centrifuged and supernatant di carded. The precipitate was washed with distilled water b dialysis for 3 hours, followed by dialysis against saline fo 24 hours. After centrifuging the resultant contents of th dialysis bag, the supernatant was removed and made u to original serum volume.

3. *Tissue Incubation*

Cerebral slices were obtained from normal adult ma 150 gm Wistar rats. These were killed by decapitation an brains removed quickly. A Stadie-Riggs slicer was used t obtain cerebral slices. Two slices were taken, the outc cortex and the immediately underlying tissue.

The incubation media was prepared and put in 25 c Erlenmeyer flasks. The incubation media contained:

(i) 2.5 cc modified Gey-Gey buffer made up to contai 2% bovine serum albumen and 300 mg of D glucos

(ii) 0.4 cc normal *or* EAE serum

Each cerebral slice was then divided into two section one part added to a flask containing normal serum and th other to a flask containing EAE serum so that there wer 4 flasks for each rat. The flasks were weighed before an after the tissue was added to determine the tissue weigh

The flasks were then incubated in a Dubinoff shaker a 38°C for three hours (unless otherwise stated).

Control experiments were run using epididymal fat pac instead of cerebral slices.

4. *Fatty Acid Output* (40)

After incubation the media in the flasks was filtere using Whatman No. 1 filter paper. A 1 cc aliquot was re moved and added to 5 cc of extraction mixture. The ex traction mixture contained 40 parts isopropanol, 10 par heptane and 1 part 1N sulphuric acid. These tubes wer then capped and shaken vigorously for one minute an allowed to stand for ten minutes. Then 3 cc of heptan followed by 2 cc of distilled water was added. After shak ing, the mixture was allowed to separate into two phase 3 cc of the upper phase was removed to a separate te tube and 1 cc of titration medium was added. This me ium contained 1 part 0.1% thymol blue in ethanol solutio with 9 parts redistilled ethanol. These tubes were kep covered since they were sensitive to CO_2 absorption.

The resultant solution was titrated against 0.02N pota sium hydroxide using a microburette. Air blown through 50% saturated solution of NaOH, was bubbled through th solution to ensure mixing.

Flasks were incubated without tissue to provide a bas line of fatty acid content in the sera alone. To determin the concentration of fatty acid, standard oleic aci 4mEq/L was titrated against the 0.02N KOH. Fatty aci output was then calculated in micro Eq/Gm of tissue.

5. *Glucose Uptake*

In some experiments a second 1 cc aliquot was taken from the reactant flasks and diluted 1:10 with physiological saline. The glucose content was determined using the Technicon Autoanalyzer. Values were calculated as mg/Gm of tissue.

EXPERIMENTS

A) Preliminary Experiments

1. *Fatty Acid Content of EAE vs Normal Serum*

Because it is an established fact that EAE serum is often very lipemic in comparison with normal serum, the fatty acid content of several different samples of sera used in experiments were compared (41). EAE serum, on the average, contained 0.2 mEq/L more than normal sera.

2. *Effect of Incubation Time*

In one experiment, brain slices were incubated with normal sera for one, two and three hours to determine the optimum incubation time. Six rats were used and divided into pairs. Each rat donated tissue to three flasks. The first cerebral slice was divided between flask No. 1 and No. 3 and the second slice between flasks No. 2 and No. 3.

The results showed that prolonged incubation steadily increased the fatty acid output. Therefore, in subsequent experiments the tissues were incubated for three hours.

B) The Effect of EAE vs Normal Serum

1. *The Effect of EAE serum on the Fatty Acid Output of Brain Slices*

Seven experiments were performed using a total of 19 rats. The sera used for these experiments were not obtained from the same sample but from several different animals. However, for purposes of analysis, they were considered the same. The results showed a significant fatty acid output increase in the presence of EAE serum as compared with normal serum with the first slice.

The glucose content of the incubation media was also determined in 10 animals and the glucose uptake of the tissue calculated. Only a rough correlation resulted, and there seemed to be no difference between the results with normal sera, and those with EAE sera.

2. *Effect of Saturated Ammonium Sulphate Precipitate of EAE Serum on the Fatty Acid Output of Brain Slices*

Four rats were divided between two experiments where crude gamma-globulin preparation was used in place of the whole serum. On inspection there seemed to be a significant difference between the results. However, when subjected to statistical analysis, there was no significant difference. Perhaps the smallness of the number of experiments explains the failure to obtain a significant result.

3. *The Effect of EAE and Normal Serum on Fat Pads*

The epididymal fat pad in the rat was excited and treated in the same manner as the brain slices in an effort to construct a control experiment. However, it all cases the fatty acid output was negligible and, therefore, not satisfactory for purposes of comparison.

4. *Effect of the Addition of Complement on the Effect of EAE on the Fatty Acid Output*

In two experiments using 8 animals in all, the effect of EAE serum was compared with normal sera in the presence and the absence of complement. The experimental set-up was like that of previous experiments except that the tissue from 4 rats had complement added and the remainder did not have complement added.

The result of the statistical analysis shows a significant interaction term between complement and sera so that it may be concluded that complement does affect the EAE effect. The addition of complement significantly increased the fatty acid output of tissue with normal sera present ($t=6.07$, P less than 0.001 with 14 d.j.).

It was also noted that the titration value of the blanks of EAE serum were elevated more than those of normal serum when complement was added. Since these values were duplicated, it is unlikely that the differences were due to technical error. The elevation was probably due to impurities in the complement, at least in part.

DISCUSSION

The incidence of disease in this laboratory was not as high as anticipated in view of the literature. The source of this difficulty may lie in the fact that the guinea pigs were of a mixed strain. Inbred strains have been reported more susceptible than mixed, with the Hartley strain being particularly susceptible (42, 43).

Control experiments might have been run using Freund's adjuvant alone as innoculum or with some tissue other than nervous tissue.

A question may arise concerning the choice of fatty acid output and glucose uptake as parameters to be measured. In a sense, this was a step in the dark. However, fatty acids are basic components of lipid compounds. Therefore, during a process of lipid breakdown, one might expect to find a release of fatty acids. Since myelin is highly lipoid, breakdown products would probably include fatty acids.

Glucose utilization is basic to, and indicative of metabolic activity. Therefore, the glucose uptake of the rat brain slices was measured to see if there was any correlation with changes in the fatty acid output.

One might also question the use of *guinea pig* serum and *rat* cerebral slices. In other words it was expected that an antibody to guinea pig spinal cord produced in guinea pigs would affect the rat cerebral tissue. Since the agent in EAE is postulated to be non-species specific but organ

specific, the species difference ideally should not alter the effect (44). In further defense, it may be recalled that Bornstein and Appel produced their serum in rabbits using guinea pig brain as antigen (38). This serum then was used to treat tissue cultures of rat cerebellum. Regardless, our experiments might well be repeated using the same species for both the serum production and tissue incubation.

The ideal tissue to be used for incubation would be white matter since it contains the most myelin. However, in these experiments cerebral slices were used; these contained more grey than white matter. The cerebrum lends itself to slicing more easily than other parts of the brain and spinal cord. Future experiments might use cerebellar or brain skin tissue if technically possible.

The results using the crude globulin fraction appeared to give a similar result as the whole serum on inspection. No significant difference was demonstrated by statistical analysis. In many ways these experiments parallel those of Bornstein. His cultures were viewed histologically for evidence of myelin breakdown whereas the present experiments approached the problem from an analytical viewpoint seeking a more quantitative means of assessing breakdown. It was expected that antigen antibody interaction would cause, in some undetermined manner, a breakdown in the lipid components of myelin. If it has been possible to isolate a fatty acid output enhancing effect on the globulin fraction, the antigen-antibody theory would have gained support.

The effects in experiments with complement were not anticipated. In the first place the addition of complement to the medium raised the titration value showing there must be impurities of an organic acid nature in the complement. One would expect the elevation to be constant in the EAE and normal serum, which it was not. Perhaps the complement is active in releasing into the serum fatty acids which were bound either to plasma proteins or in the plasma lipids. The lipid content of EAE serum as mentioned before is elevated. Complement also increased the net fatty acid output of brain slices when normal serum is present. This action of complement was not expected and is difficult to explain. Alternatively, its presence seemed to decrease the effect of EAE serum. A significant interaction term between the serum and complement in the statistical analysis suggests that complement does alter the effect of EAE. Further experimental work needs to be done to clarify this action of complement.

SUMMARY AND CONCLUSIONS

1. The literature concerning the basic principles of myelin and demyelination was reviewed briefly.

2. The background of Experimental Allergic Encephalomyelitis with particular emphasis on its immunological nature was discussed.

3. The experimental procedure, which enabled cerebral slices to be incubated with the EAE serum and the fatty acid output and glucose uptake of these slices determined, was described.

4. From experimental results, it may be concluded that using this procedure the EAE serum stimulates fatty acid output from the first cerebral slice; however, the effect on the second slice is marginal, if present at all.

5. When glucose uptake and fatty acid output were compared, only a rough correlation between them was present. Increase in glucose uptake is accompanied by increase in fatty acid output.

6. Experiments with complement showed that its presence increases the fatty acid output of rat cerebral slices. It also alters the effect of the EAE serum.

7. In the course of the discussion, some improvements in experimental procedure were proposed.

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Abstracts

Cesarian Section on the Dead and the Critically Injured.

Vitsky, Meyer: Am. J. Obs. Gyn., 90:17-24, Sept. (1964)

A brief discussion of medical, legal and moral aspects of post mortem cesarian section. The author urges fixed policies of procedure to prevent unwarranted waste of time when such operations are indicated.

Delivery should be performed within minutes of the mother's death using any sharp instrument and sterile precautions where possible. Best prognosis is offered infants of at least 28 weeks' gestation and following sudden, rapid maternal death.

Legally, relatives' consent is not needed and the physician is morally obligated to attempt to save the child.

Several case histories are presented supporting the adoption of standardized emergency procedures for post mortem cesarean sections to reduce unnecessary fetal death.

M.T.

Management of Hyperpyrexia in Children

Fruthaler, G.J., Tilden, T.: Postgrad. Med., 35:643-6, June (1964)

A presentation of practical points in the management of febrile children. High fever is not considered an emergency, is not necessarily harmful and treatment can be deferred until a diagnosis is made. 90% of acute febrile illnesses have no important consequences and rare, serious diseases require long-term therapy. A careful history and clinical examination should be accompanied by urinalysis, urine culture, chest x-ray, and, sometimes, a blood culture.

Therapeutic measures include: (1) body sponging, which must be governed by the patient's comfort and not the thermometer; (2) salicylates, the safest, least expensive and most effective antipyretic and (3) rarely, antibiotics. When these are indicated, a single agent against a specific pathogen must be given in full therapeutic dosage.

M.T.

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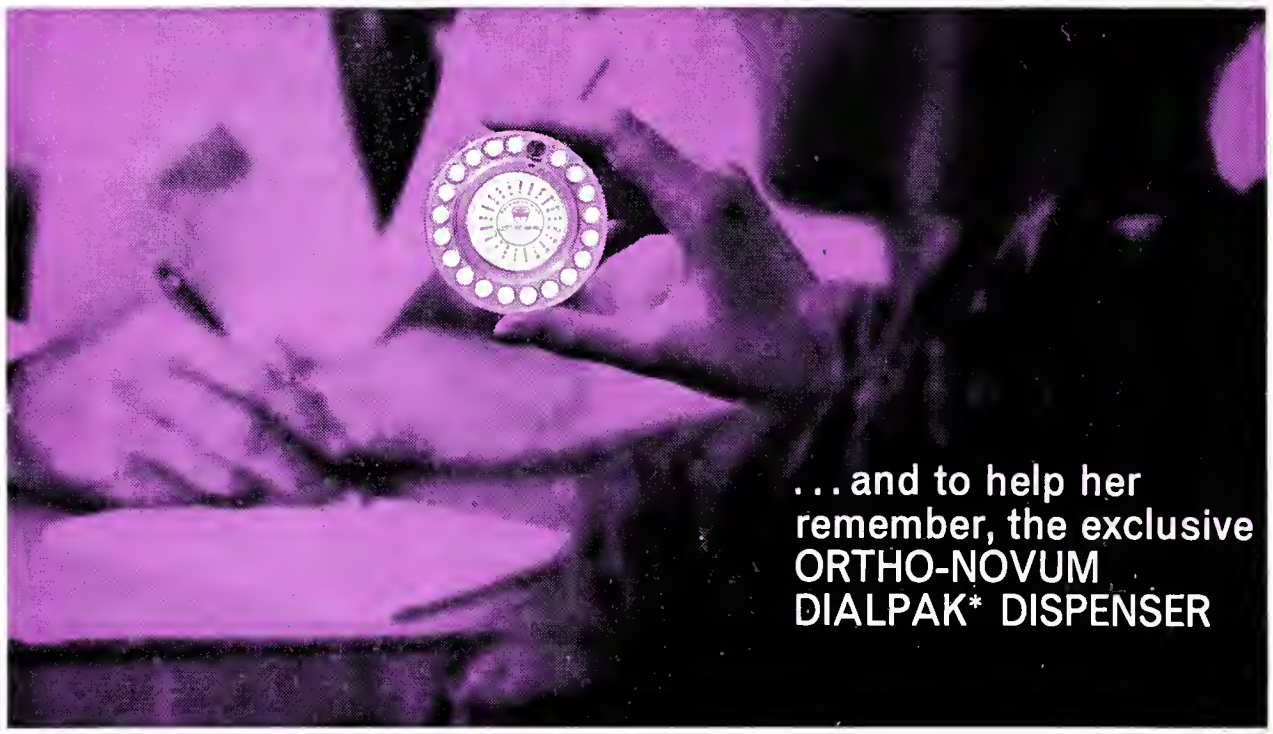

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A little learning is a dangerous thing;
Drink Deep, or taste not the Pierian spring:
There shallow draughts intoxicate the brain,
And drinking largely sobers us again.
Fired at first sight with what the Muse imparts,
In fearless youth we tempt the height of arts,
While from the bounded level of our mind,
Short views we take, nor see the lengths behind
But more advanced, behold with strange surprise
New distant scenes of endless science rise!
So pleased at first the towering Alps we try,
Mount o'er the vales, and seem to tread the sky,
The eternal snows appear already passed,
And the first clouds and mountains seem the last:
But, those attained, we tremble to survey
The growing labours of the lengthened way
The increasing prospect tires our wandering eyes,
Hills peep o'er hills, and Alps on Alps arise.

ALEXANDER POPE
An Essay on Criticism

HISTORY

A knowledge of the heritage or history of a problem in any field of investigation is a fundamental prerequisite to our appreciation of its significance today. In this section only some of the more significant events that have evolved from basic research into the problem of blood coagulation and its control can be considered.

The whole subject began when William Harvey published his *De Motu Cordis* in 1628. Lister's paper (1) *On the Coagulation of Blood,* in June, 1863, is a classic paper in the field. In 1905, Morawitz (2) postulated what is now the classical theory of blood coagulation. Four factors were required: thromboplastin, calcium, prothrombin and fibrinogen. Nolf (3) separated blood coagulation into three distinct phases. He also described the lysis of fibrin by an enzyme of plasma origin he called "plasmin".

In 1916, McLean (4), a medical student, published a paper on the thromboplastic activities of cephalins. In the last paragraph of his paper, he mentions the "heparphosphatides" which were anticoagulants rather than procoagulants. Howell and Holt followed McLean's work and prepared "heparin" from lymph glands.

In 1922, a very brief and hurried paper by Schofield (5) appeared in the contemporary veterinary journals. It described a disease in cattle simulating a haemorrhagic septicaemia but due to the feeding of spoiled sweet clover. A more detailed report appeared in 1924, but it was necessary to await the work of Link et al. before dicoumarol was isolated.

In 1936, Best et al. (6) reported the first successful clinical trials with heparin as prepared and purified by Charles and Scott. (7)

THE CLOTTING FACTORS

Nomenclature for Blood Coagulation Factors

Factor	*Synonym*
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Accelerator globulin, labile factor
VI	Not generally accepted today
VII	Proconvertin, stable factor
VIII	Antihaemophilic globulin AHG
IX	Plasma thromboplastin component Christmas factor, PTC
X	Stuart-Prower Factor
XI	Plasma thromboplastin antecedent PTA
XII	Hageman Factor

Factor I — Fibrinogen

Fibrinogen is the plasma protein clotted by thrombin. It is normally present in the blood in concentrations of 300 - 500 mgm%. The fibrinogen molecule has a molecular weight of 330,000 and contains six chains of amino acids. On electron microscopy, the molecule appears as three linked globules with the terminal globules being slightly larger than the central one. The central globule may be a fibrin stabilizing factor which contaminates most fibrinogen preparations. A congenital hypofibrinogenemia has been described by several authors the most recent of which being Hasselback et al. (8) in 1963. This disease presents as a relatively mild bleeding tendency inherited as an autosomal dominant with variable penetrance; the fibrinogen levels vary from 58 - 158 mgm%.

*Precis of winning essay, the Charles E. Frosst Scholarship 1964.

Factor II — Prothrombin

Prothrombin is the plasma protein which is converted to thrombin — the natural coagulant of fibrinogen. It is formed in the liver and present in the blood at concentrations of about 20 mgm%. Prothrombin has a molecular weight of 68,900 and behaves electrophoretically as an alpha 2-globulin. It contains carbohydrate possibly derived from factor VII, a glycoprotein, which contaminates most preparations of prothrombin. The one- and two-stage prothrombin and the prothrombin consumption tests are currently used to assay prothrombin activity.

Some of the most exciting work in blood coagulation has been done in Seegers' laboratory. They have isolated several derivatives of prothrombin which have the activity of factors VII, IX and thrombin. The diversification is derived from the variations at which the prothrombin molecule is split away from active centres. Since these autoprothrombins are derivatives of prothrombin, one would expect them to decrease along with prothrombin during Dicoumarol therapy and this indeed is the case.

Thrombin

Thrombin is the natural coagulant of fibrinogen which appears in the blood following the activation of the clotting mechanism. There are probably several different molecules with thrombin activity. Thrombin acts on fibrinogen as a proteolytic enzyme splitting arginyl-glycine bonds to yield four fibrinopeptides. These monomers have lost the negative electric charge of the fibrinogen molecule and now can react by purely physico-chemical principles to produce the fibrin polymer. This product is soluble in 5 M urea. Laki and Lorand (9) have isolated a thermolabile component of plasma which possesses protease activity and stabilizes the fibrin network. The fibrin is now insoluble because of disulphide cross-linkages which leads to a three dimensional cross-linked structure.

Duckert et al. (10) have described a haemorrhagic diathesis caused by a congenital deficiency of FSF. The blood clots of the patient and two relatives began to dissolve in 5 M urea within one hour. The patients presented with slow poor wound healing, profuse and prolonged bleeding following trauma but no spontaneous joint bleeding.

Factor III — Thromboplastin

With more recent advances in the study of prothrombin activation, thromboplastin can no longer be conceived as a "factor" but rather an "activity". It is a hypothetical concept used to describe the various environmental situations in which prothrombin activation occurs. However, the thromboplastin generation test is still a very useful clinical tool. In this test, aluminum hydroxide treated plasma, a platelet suspension and serum diluted 1:10 with isotonic saline are used. These factors are required for prothrombin activation. The patient's blood is used to replace one of the three components. For example, in patients with haemophilia A the plasma component is deficient (factor VIII), and in haemophilia B the serum component yields a prolonged time.

Factor IV — Calcium

Calcium takes part in several reactions in the clottir mechanism. In some, it acts as a co-factor to an enzym In others, it contributes to the structural integrity of th reaction product. The role of calcium as it pertains t the function of other clotting factors is mentioned in sul sequent sections.

Factor V — Labile Factor or Accelerator Globulin

In 1947, Owren (11) described a patient with a haemor hagic diathesis characterized by a prolonged whole bloo clotting time and prothrombin time. He found the pro thrombin time could be shortened by the addition o plasma from which the prothrombin had been removed He called the factor present in normal plasma Factor V A deficiency of factor V is very rare and the disease ha been called parahaemophilia. A circulating anticoagulan against Factor V has been reported.

Factor VI

Factor VI was introduced by Owren as an activate form of Factor V but it is no longer accepted today.

Factor VII — Proconvertin or Stable Factor

In 1948, Owren found that the addition of serum t the plasma of patients receiving Dicoumarol therapy woul shorten the one-stage prothrombin time. The factor coul not be prothrombin or Factor V since neither were preser in serum. Alexander et al, then went on to characteriz the factor physically and chemically and described a con genital haemorrhagic diathesis characterized by a pro longed prothrombin time but a normal whole blood clottin time and prothrombin consumption. The prothrombin tim could be shortened by the addition of Russel's vipe venom instead of tissue extract.

Factor VIII — Antihaemophilic Globulin

Factor VIII is the plasma protein which corrects th clotting defect and haemostatic defect in true haemophili This is a sex-linked disease occurring in males and cha acterized by a normal one-stage prothrombin time but pro longed whole blood clotting time and deficient prothron bin consumption. The factor is relatively stable on storin Factor VIII is not found in serum, and studies with fluo escein labelled factor VIII have shown that this factor bound to the fibrin of wounds. Tissue necrosis, exercise an adrenalin seem to reduce the plasma levels of factor VII This may account for the disastrous haemorrhagic cris seen in haemophilia. A circulating anticoagulant again factor VIII has been described.

Factor IX — Christmas Factor; Plasma Thromboplast Components

Pavlovsky (12), in 1944, noted that if the blood of tw patients with clinically similar haemophilia was mixed th whole blood clotting time was shortened. Eight years lat

Biggs (13), using the thromboplastin generation test which he had described, differentiated this factor from factor VIII. She called it Christmas factor after her patient. Factor IX appears to be antigenic and several authors have reported circulating anticoagulants against this factor. Its metabolism appears dependent on hepatic function and adequate vitamin K. Its level is depressed by coumarin drugs.

Factor X — Stuart-Prower Factor

Hougie et al. (14), in 1957, described this factor and named it after their first patient. Mr. Stuart was first thought to have a factor VII deficiency but re-study showed that the plasma of a patient with a classical factor VII deficiency would correct the prolonged prothrombin time of Mr. Stuart's plasma. The disease is inherited as an autosomal recessive. Factor X is found both in plasma and serum, and is depressed by coumarin therapy at a slower rate than is factor VII.

Factors XI and XII — The Contact System

Rosenthal et al. (15) described a congenital haemorrhagic disease with a normal prothrombin time but prolonged whole blood clotting time and deficient prothrombin consumption. The thromboplastin generation test was abnormal only if both the patient's plasma and serum were used simultaneously. This was the plasma thromboplastin antecedent.

Ratanoff et al. (16) described a clotting factor which was activated by glass contact and described a bleeding disorder caused by a deficiency of the "Hageman factor". The haemostatic defect is relatively mild perhaps because of a concomitant decrease in the fibrinolytic activity of plasma. Jürgens (17) has described in a most interesting paper, the kinetics of the early phases of contact activation.

Platelet Factors

It has been known for a long time that platelet deficient plasma created an abnormality in the thromboplastin generation test. Phosphatidyl ethanolamines and serine phosphatides can replace platelets in this test. Johnson isolated platelet factor III from the granules in platelets and showed, with Seegers, that it was crucial to prothrombin activation. Platelet factor IV is found in the hyalomere of platelets and appears to have antiheparin activity. It is reduced in patients receiving anticoagulants and in patients with cirrhosis of the liver or chronic uremia.

The Natural and Spontaneous Anticoagulants

Having reflected on the large number of factors which initiate, promote and accelerate the clotting of blood, one wonders how blood ever remains in a fluid state! The natural anticoagulants are generally considered to have antithrombin, antithromboplastin or combined activity. There is another species of anticoagulants which appears spontaneously in certain disease states. There are six known antithrombins with very different activities. Antithromboplastin is found in undiluted plasma but is much less effective if diluted. There has not been a great deal of work on this substance. Spontaneous anticoagulants have been reported in: anaphylactic shock, chronic leukemia, hepatic cirrhosis, ITP, pregnancy, DLE, multiple myeloma and several other conditions.

THROMBOGENESIS

This is a whole field separate from the main stream of thought of this essay. However, some of the essential findings of this field of research must be correlated with that of blood coagulation. Essentially, there are three stages in thrombogenesis: 1) some local vascular defect whether it be endothelial in origin or an alteration in blood flow, 2) a contact activating system involving platelets and several other factors and 3) prothrombin activation. An enormous amount of work has been done on the first stage of this process as it pertains to atherosclerosis. It is known that a change in the surface charge of the endothelium will produce "a current of injury" which predisposes platelets to aggregation. Cell injury also effects a release of ADP which may cause platelets to adhere to surfaces. These factors may be causally related. Alterations in the laminar flow of blood are also important predisposing factors. Following this first stage, the contact activation system comes into play. Waaler has isolated an activation product containing almost solely factors XI and XII. Wessler has presented evidence for a serum thrombotic accelerator which may be responsible for the initial local hypercoagulable state. Jürgens has postulated that activated factor XII on a wettable surface may react directly with prothrombin producing thrombin which causes an increase in platelet adhesiveness and thus platelet agglutination. This reaction does not require calcium.

Now the most important stage in thrombogenesis, as far as our understanding of the physiological basis of anticoagulant therapy is concerned, is the third stage. In 1955, a concept arose in Oxford that there were two separate systems for prothrombin activation. They were found in the intravascular and extravascular compartments, and were called the intrinsic and extrinsic prothrombin activator systems. Both systems were required for physiological haemostasis. The "trigger" mechanism in the intrinsic system was the contact system while in the extrinsic system it was tissue extract activity. The known components of these systems are tabled below:

Intrinsic System	*Extrinsic System*
Platelets	Tissue factor
Factor V	Factor V
Factor VIII	Factor VII
Factor IX	Factor X
Factor X	Calcium
Calcium	

The earliest events in the intrinsic system involve the activation of factor XII by contact. Factor XII generates thrombin from prothrombin which increases platelet adhesiveness perhaps by causing a release of ADP from the cells. The platelets then contract releasing their granules of active phospholipid. Activated factor XII reacts with factor XI yielding an intermediate reaction product. This reaction is not influenced by heparin. This "prephase" system activates factor IX which in turn reacts with factor XIII. Factor X is an enzyme in this reaction and calcium is required. Product I then reacts with factor V which has had its molecular configuration altered by platelet phospholipid. The reaction product, probably by proteolysis, converts prothrombin to thrombin and then proceeds rapidly perhaps by autocatalytic reactions. Now in this set of reactions factors V, VIII and IX act as substrates and one would expect them to disappear during coagulation. This is indeed found with factors V and VIII. However, factor IX may actually increase and this may represent Seegers' autoprothrombin II being generated from prothrombin yielding product I.

In the extrinsic activator system, release of phospholipids and/or certain lipoproteins from damaged cells activates tissue thromboplastin. This reacts with factor X to produce an intermediate reaction product. Factor VII catalyses this reaction. This extrinsic reaction product activates factor V to form the extrinsic prothrombin activator which converts prothrombin to thrombin. One obvious criticism of this mechanism is that factor X acts as a substrate in this system but as an enzyme in the intrinsic system. This problem has not yet been answered.

The action of thrombin on fibrinogen was discussed previously.

It must be recalled that prothrombin activation takes place in a milieu of natural anticoagulants, fibrinolysins and antifibrinolysins. Activity in one of these systems may be reflected in other systems. It is the sum total of activity and counter-activity that is reflected in the one empirical fact—the clotting of blood.

THE ANTICOAGULANT DRUGS

The anticoagulant drugs may be considered in two broad categories: the oral anticoagulants and heparin. The oral anticoagulants may then be subdivided into coumarin and indanedione derivatives.

The oral anticoagulants are absorbed well from the gastro-intestinal tract. The plasma peak levels differ between these two types of oral anticoagulants but the "prothrombin response" to these drugs occurs at about the same time. The delay in the prolongation of the one-stage prothrombin time is probably related to the half life of circulating clotting factors. Biggs and Douglas have shown that the one-stage clotting times in normal plasma and prothrombin deficient plasma were very similar. The prothrombin content of the latter was only ten percent of normal. However, coumarin treated plasma had a long one-stage clotting time though the prothrombin content was sixty-eight percent. Thus coumarin drugs must affect other clotting factors as well as prothrombin. Factor VII deficiency develops rapidly and factor X deficiency follows later. The contact activating and antithrombin systems remain intact. Factor IX is also depressed with oral anticoagulants. The reduction of all these factors is relative to their turnover rates. Wessler emphasizes that oral anticoagulants do not depress his STA factor as does heparin.

Heparin is a mucopolysaccharide with a remarkable high concentration of sulphate groups which appear to be crucial to its anticoagulant activity; heparin is thus strongly negatively charged. It is water soluble and can react with protein producing a visible precipitate at the physiological pH. Heparin can form soluble complexes with most of the beta-globulins and possibly some of the alpha-globulins and fibrinogen. Many of the clotting factors are also globulins. Douglas (18) points out that heparin interferes with blood coagulation at two points:

a) the thrombin—fibrinogen reaction and

b) the intrinsic prothrombin activator system.

Heparin requires a lipoprotein co-factor for its activity. Monkhouse and others have presented evidence that this co-factor is antithrombin. O'Brien (19) feels that the acidic groups of heparin react with the basic groups of plasma A-globulins. The reaction product is soluble and the reaction is reversible. An early reaction between heparin and factor IX probably accounts for at least part of its anticoagulant activity in the intrinsic prothrombin activator system.

Anticoagulation is achieved when we have minimized the chance of blood coagulation without depriving the patient of his normal haemostatic mechanisms. This level is generally defined empirically in terms of one or more of a variety of tests used to control anticoagulant therapy.

The criterion for adequate heparinization is a clotting time of two to three times that of a normal control. The dilemma is found in the control of oral anticoagulants. The one-stage prothrombin time reflects factor VII and factor X depression as well as prothrombin deficiency. In 1959, Owren (20) pointed out that prothrombin, factors IX and X are depressed in the intrinsic system and factors VII and X in the extrinsic system with coumarin drugs. He introduced his "Thrombotest" which measures the effect of oral anticoagulants on both the intrinsic and extrinsic systems. The test was rapid but relatively expensive. However, MacMillan, Brown and Watt (21) have described a simple method of doing prothrombin times using capillary blood. The test is extremely useful and inexpensive and is excellent for out-patient control.

INDICATIONS FOR ANTICOAGULANT THERAPY

It seemed appropriate that at the end of this monograph I should conclude my discussion of anticoagulants on this topic. However, on reviewing the literature, I was able to find at least three authors defending each side of the argument for anticoagulants in every conceivable indication I could think of. In many well controlled series, the results were equivocal and where results appeared definitive the clinical trial was poorly controlled. The problem is further complicated by reports of a hypercoagulability state on withdrawal.

Anticoagulants do not alter the pathology of the vascular bed, nor do they, at therapeutic levels, affect the early phases of thrombosis. They do alter markedly the third step in thrombogenesis — the formation of a red thrombus and its propagation. Therefore, anticoagulant drugs would be expected to be most effective in conditions where extension of a clot is threatened. The anticoagulant must get to the area of thrombosis or potential thrombosis. Thus, a situation in which there is a collateral circulation is optimal for a therapeutic effect. Conditions such as recurrent or migratory thrombophlebitis, phlebothrombosis and atrial fibrillation are strong indications for such therapy. In those conditions where there is thrombosis of an end artery such as myocardial infarction, cerebral thrombosis or complete thrombosis of the retinal vessels anticoagulants achieve but little. The MRC trial revealed equivocal results. MacMillan and Brown (21) could show no real value for anticoagulants in these situations. Some of the better trials are included in the bibliography for the readers' perusal.

FIBRINOLYSIS

The activity of the body's fibrinolytic and antifibrinolytic systems have a direct bearing on the physiology of the procoagulants and anticoagulants. Blood coagulation must be viewed as a dynamic process in which these four systems interact. A reaction sequence in one system influences the activity of the other systems. What is described here is a brief sketch of fibrinolytic mechanisms and the reader is referred to the writings of Fletcher et al. on the subject.

Normal blood contains plasminogen, a proteinase, which in the presence of certain activating substances is capable of hydrolyzing the internal peptide bonds of the protein fibrin. Plasminogen activators are found in the plasma (plasma activator), urine (urokinase) and tissues (tissue activator). Plasmin is the active proteolytic enzyme which will degrade fibrin, fibrinogen, factors V and VIII. Staphylokinase and streptokinase, derived from bacteria, and the Hageman factor may also play some indirect role in plasminogen activation. When clotting occurs the thrombus will contain a substantial quantity of plasminogen in intimate spatial relationship to the fibrin fibrils. This plasminogen is said to exist in the gel phase whereas plasma plasminogen is in the soluble phase. Plasminogen activation in the soluble phase has no effect on plasma substrates because it is inhibited by plasma antiplasmin. Plasminogen activation in the gel phase, because of its intimate spatial relationship to its substrate, fibrin, will produce thrombolysis independent of plasma inhibitors. Fletcher, Sherry et al (22) call this the dual phase concept of plasminogen activation.

Streptokinase affects the soluble phase plasminogen and there is no change in thrombolytic activity until the fibrinogen level has decreased by thirty percent. This is seen only if the drug is administered rapidly so that all the plasmin is not inhibited by plasma antiplasmin. Urokinase, on the other hand, is a predominantly gel phase plasminogen activator. On infusion, there is an increase in thrombolytic activity and only a slight change in plasma plasminogen. In fibrinolytic therapy, we are concerned with gel phase activation. This presents a great difficulty in attempting to monitor therapy since plasma levels may be unaffected. This problem is yet to be answered.

Therapeutic thrombolysis is still in its infancy. Standardization of agents is still not satisfactory. Laboratory control is still inadequate and clinical trials have been largely unsuccessful. Streptokinase toxicity is still a problem.

An interesting off-spring of fibrinolytic research is the studies with epsilon amino-caproic acid which is excreted and concentrated in the urine; it has been found to decrease the post-operative haematuria following transurethral and supra pubic prostatectomies. Urokinase apparently lyses blood clots in the urogenital tract and causes the haematuria; epsilon amino-caproic acid inhibits this activity.

This whole area of investigation requires much more intensive research both at the biochemical and clinical levels. The rôle of fibrinolysins in coagulation physiology has not been clearly elucidated. The clinical import of these agents has yet to be felt.

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Abstracts

Penicillin Hypersensitivity in Patients with Bacterial Endocarditis

Grieco, M.H., Dubin, M.R., Robinson, J.L., Schwartz, M.J.: Ani. Int. Med., 60:204-216, Feb. (1964)

The authors present a reviow of 100 patients with bacterial endocarditis, 10 of whom exhibited penicillin hypersensitivity. Two types of reaction were seen: (1) an immediate response, either as an anaphylactoid reaction or serum sickness, associated with previous penicillin sensitization; or (2) delayed allergic reactions usually resulting in skin rashes and often associated with previous fungus infection. All forms of penicillin are immunologically similar and an intradermal skin test is suggested to detect allergy.

Therapeutic measures in penicillin-sensitive patients with bacterial endocarditis include: (1) penicillin desensitization, a potentially fatal procedure; (2) antihistamines, showing variable success; or (3) steroids, effective only against serum sickness but ineffective in anaphylactic reactions. Alternatively, erythromycin, streptomycin, ristocetin or vancomycin may be beneficial and only failure of response to these justifies penicillin desensitization.

M.T.

Major Abdominal Surgery in Patients Over 70 Years of Age

Robins, R.E., Middleton, A.G.: Can. J. Surg., 7:129-133, Apr. (1964)

A survey of 342 male patients over 70 undergoing major, abdominal, elective and emergency surgical procedures. Pre-existing cardiovascular and genitourinary diseases were not associated with post-operative complications but respiratory diseases caused a doubling of these, usually atelectasis or pneumonia. Pulmonary complications were the commonest cause of post-operative death.

The mortality rate was 12.5%, half being due to medical complications. Infection was the chief fatal surgical problem. There was a significant increase in fatal pulmonary embolism associated with post-operative thrombophlebitis.

Morbidity and mortality can be reduced significantly by post-operative breathing exercises, by use of local block anesthesia, by efforts to reduce wound infection and by minimal urinary contamination in necessary catheterization.

M.T.

Medical Muses

Hippocrates

(This is the first in a series of six short articles about famous personalities in Medicine and the era in which they practiced. Special reference will be given to their contributions to modern medicine.)

It should only be proper to start with a short biography of the Father of Medicine, Hippocrates. It has been maintained however, that Imnotep, physician and adviser to King Zoser of Egypt about 2980 B.C., should supplant Hippocrates as the Father of Medicine. Although he achieved notable success as a politician and as an architect (he designed the Step Pyramid of Sakkarah), his prowess as a physician in Medicine is little known even though he was worshipped as a full deity, the God of Medicine.

Hippocrates, born in 460 B.C. in the island of Cos, gave birth to scientific medicine and dispelled any attempts to explain disease as punishment sent by the Gods. Sound, accurate observation and logical reasoning, which was the foundation of Hippocratic medicine, was in sharp contrast to the superstition and empiricism of the early Greek and primitive medicine.

Medicine of the early Greeks was in the hands of the priests of Aesculepius, whose temples of healing were located about the country side. Aesculepius, who probably lived around 1250 B.C. acquired, so legend says, great powers of healing and was even said to give life to the dead. Pluto, God of the Underworld, complained to Zeus that not enough souls were reaching Hades because of Aesculepius's healing power. Zeus, in compliance, slew Aesculepius with a thunderbolt.

The symbol of Aesculepius — the caduceus - the two snakes twined on a staff — was survived and is the well recognized medical emblem. The world hygiene has been derived from Hygeia, the daughter of Aesculepius. Hippocrates has been said to have been a descent of Aesculepius.

Corpus Hippocratium, a collection of more than 100 books, give a complete account of the method of Hippocrates. Great attention was given to the scientific method. His ability to make accurate observation, to rewrite precise accounts of case histories and to search for a natural cause laid the foundation for medical growth and development. The Oath of Hippocrates which lays down the ethics of medical conduct is still, in principle, applicable today. Also it is of interest to note the reference to early Greek medicine in the opening lines: "I swear by Apollo, the physician, and Aesculepius and Hygeia and Panacea. . . ."

Hippocrates believed in ascribing a disease process to natural causes. He states in his book "The Sacred Disease" (presumably epilepsy): "It is not in my opinion any more divine or more sacred than other diseases, but has a natural cause". Frequent references are made in his books about the relationship of environment, airs, winds, water supply and nature of the soil to disease. Plato was later to elaborate the humoral pathology of Hippocrates: the world was composed of four elements, air, earth, fire and water.

One of the greatest works of Hippocrates was the book *Prognostic.* In the preface, it stated "I hold that it is an excellent thing for the physician to practice forecasting . . . he will carry out treatment best if he knows beforehand from the present symptoms what will take place later". This statement would necessarily presume a detailed knowledge of the signs, symptoms and an understanding of the natural history of disease. The hallmark of medicine practiced by Hippocrates!

Hippocrates died in Thessaly but at what age is uncertain. This varies from 85 to 109 years. However, and regrettably so, the degree of development of scientific medicine was not to be again attained for centuries.

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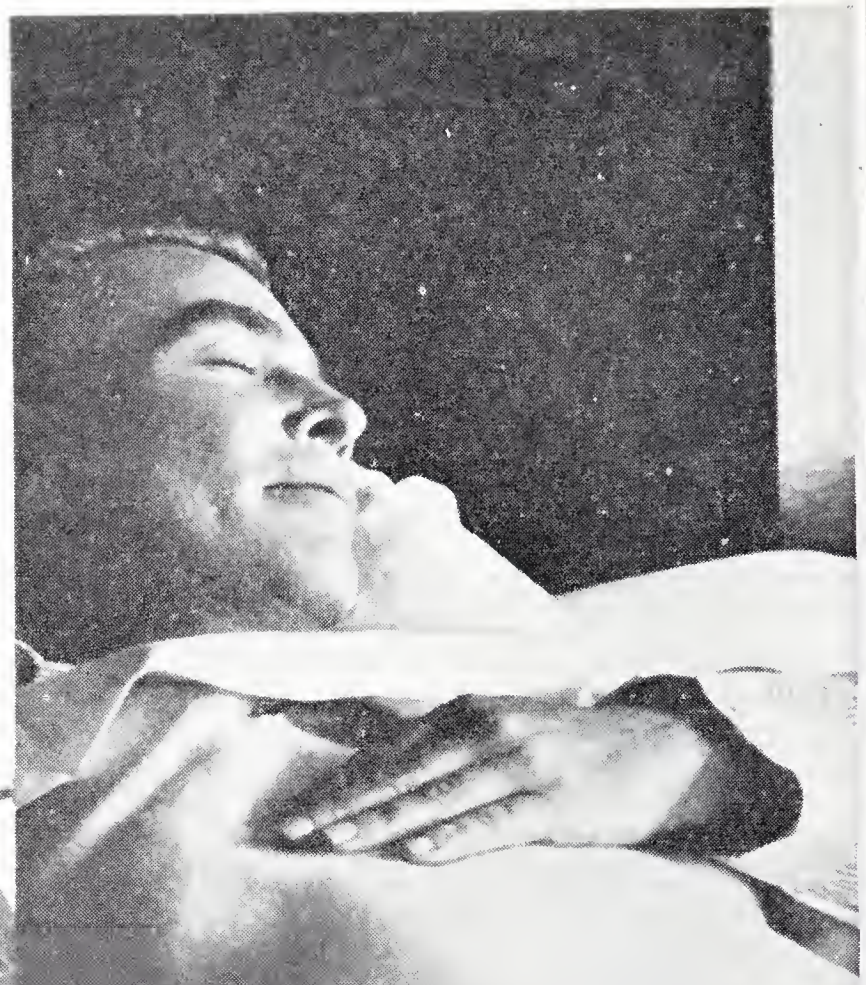

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The Influences of Group and Independent General Practice on Patient Care

E. M. Sellers 6T5

Preliminary Report on a Comparative Study in Ontario

BACKGROUND

On December 14, 1963, the College of General Practice (1) announced the establishment of Family Practice Awards. These awards were made available to undergraduates and physicians for research in family practice. Suggested topics for investigation were the epidemiology of family practice, the role of the family physician in the community, the attitudes of medical students to family practice, and the family physician and medical education. The selection and design of a project was left entirely to the judgment of the applicant. From these suggested topics, six studies have sprung (2).

The findings and suggestions of K. F. Clute in "The General Practitioner" (3) prompted the author to design a study investigating "The Advantages and Disadvantages of Group versus Independent Practice". Studies in the past have often been oriented towards doctor advantages and disadvantages. This study was specifically designed with patient benefits in mind.

In order to determine patient benefits, it is necessary to study patterns of medical practice and the resulting quality of care, for we are in a period where medical facilities and personnel are unable to keep abreast of patient demands, and inefficient use of existing facilities has serious economic and general health implications.

At present there are three ways to study medical care (4). It is possible to i) directly observe the practising physician ("observational studies"), ii) survey the records of management of patients ("record studies"), or iii) study the results of treatment ("end-result studies"; e.g., surgical successes versus failures). Of these methods, "observational studies", while apparently the most direct and scientific approach, also pose the most difficult problems in sampling, observer bias, cost, time and the necessity of employing highly qualified medical personnel. The evaluation of such a study, conducted in a limited area, poses further problems in terms of general application.

A number of "observational studies" have been done on general practice. In Britain, studies were published by Collings (5), Hadfield (6), and Taylor (7). In the United States, "A Study of North Carolina General Practice" by Peterson et al (8), is the classical paper, and forms much of the basis for evaluating general practice in North America. In Canada, Clute's "The General Practitioner" (3) is the only comparable study. Both of the latter two publications were able to relate performance in medical school, amount of residency and internship to the improved quality of medical care. Because of the problems outlined above, neither of these studies has been free from criticism.

Today, with an ever decreasing number of general practitioners (9), the trend to specialization, the introduction of health care insurance, the accent on preventive medicine and the increasing expense of newer diagnostic methods, patterns of practice become almost as important as the quality of medical care itself. Team effort as a pattern of practice is one solution to meeting these changing times. There is a marked trend toward this form of practice in North America. A United States Public Health Survey in 1959 (10) indicated that the number of groups and participating physicians had increased over 300 per cent since 1946. This represents 1 in every 10 physicians in active practice in 1959. Of the 1950 graduates in the United States, 29% of those in general practice work in partnership or group practice (11). There is no reason to suppose that such a trend does not exist in Canada.

Team effort may exist in many forms: hospital group, partnership, multi-specialist clinic, specialist-general practitioner group, and less formal arrangements among independent practitioners. Yet to be solved is whether these arrangements are in response to the pressures of providing economic volume care, or are in fact in the interests of providing the best medical care in the best context.

The advantages and disadvantages of group practice for the doctor have been presented (3,8,16); however, the impact of the pattern of non-hospital group practice on the health of the community has been virtually untouched, and is a field of research which merits investigation. Silver (12), in his study of a specialist group, was able to demonstrate an improvement in the health, housing and nutritional levels of a random patient sample over matched controls. In the United States, other studies on hospital and pre-paid, multi-specialist groups have been done (13). Because no "end-result studies" have been done in Canada, we are not yet in a position to say "the advantages of group practice so far outweigh the disadvantages that the programme should offer such inducements that a larger proportion of physicians provide their services through group practice clinics" (14).

What has been discovered comparing the quality of care in groups and in independent practice? In this study,

a group was defined as "a formal association of three or more physicians providing services in more than one medical field or specialty, with income from medical practice pooled and redistributed to the members according to some prearranged plan" (15). Despite the fact that Clute and Peterson use the term "group" to mean an association of two or more doctors, their results have an important bearing on this study. Peterson's findings are summarized:

1) the qualitative rank for "group" physicians is 3.4, versus 2.5 for "solo" ($P = 0.01$);

2) "group" doctors had better academic records;

3) "group" doctors had longer internships.

Peterson concludes that while group practice appears to have a favourable effect on the quality of medical care, it is difficult to say which is cause and which effect. Do good physicians enter group practice or do they become good under the influence of this form of practice?

Clute, however, was not able to demonstrate any difference between the mean scores obtained by "group" and independent doctors. These findings are more pertinent as his study was done in Ontario.

There are relatively few facts directly comparing independent and group practice. In the realm of speculation, however, many theoretical differences exist. This study endeavoured to provide some facts relating to these two types of practice in Ontario.

METHOD

The purpose of this study was to record and compare by the "observational method" the management of patients seen by independent and group practitioners, and from these comparisons determine if differences could be interpreted as patient advantages or disadvantages. In order to reduce observer bias to a minimum, parameters were selected which did not require a judgment of quality.

The study was conducted during the summer months in the Fort William—Port Arthur area. A week was spent with each of four group and four independent practitioners whose practices were considered by an informal board of specialists to be representative of good medical care. The physicians were accompanied on all visits in the hospital, in the office and to the patients' homes.

The two types of practice were compared on the following factors: sex and age distribution, diagnoses made, site of visits, numbers of new and returning patients, the volume of preventive medicine, the use of diagnostic facilities and diagnostic X-ray, the number of special procedures done, pattern of drug usage, the rate and patterns of consultation and referral, personal interaction between doctor and patient, medical explanation given, and t doctors' impressions of what advantages their type practice offered to patients.

The results of this study will be presented in the futu Preliminary evaluation emphasizes the *similarity* betwe these two types of practice.

Research in general practice, neglected in the past, rightfully coming into its own. There are many importa problems in this field to be solved. For the student, b sides being an excellent research opportunity, doing study on general practice provides an opportunity for a extended preceptorship. The time spent with family pra titioners is a time to learn about the common problen of practice and about medical economics. However, mo important, it should be a time in which views of the pra tice of medicine mature and are related to the student present and future training.

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Recent Advances

Mammography

X-ray examination of the breast, although described in he literature 35 years ago (9), only now is being recogized as a useful diagnostic technique in the investigation f breast disease. As in the case of other radiographic udies, mammography is not designed to replace adequate hysical examination, but rather to act as an aid in reachng a diagnosis (10). Regardless of the interpretation of mammogram, the surgical dictum to biopsy any questionable nodule should be followed (8).

ETHOD

A low voltage and high milliamperage unfiltered -ray beam and fine grain film without intensifying creens, are utilized to make exposures of the breast in hree planes; craniocaudal, mediolateral and axillary (2). he views must be highly coned down and preferably aken with a special cone flattened on one side. The radiogaphy equipment in the average radiological department ill fulfill the necessary requirements.

ADIOLOGICAL MORPHOLOGY
F BREAST LESIONS (1,2)

Benign lesions are characterized radiologically by:

. Rounded or smoothly lobulated shape;

. Homogeneous density;

. Thin surrounding radiolucency due to fat pushed aside by an expanding non-invading tumour;

. No secondary changes of skin, veins, or axillary nodes;

. Coarse and dense calcifications in some cases of fibroadenoma, plasma cell mastitis, calcifying cysts, arteriosclerosis, and fat necrosis.

Malignant lesions are characterized by:

. Irregular or round shape;

. Density which is greater in the centre of the nodule;

. Irregular spiculated border with evidence of invasion of nearby tissue;

. Secondary changes;
a. skin thickening (either diffuse or localized),
b. nipple or skin retraction,
c. venous engorgement,
d. axillary node involvement;

5. Calcifications fine and dot-like in appearance are important signs in the diagnosis of cancer. They are widely dispersed in ductal carcinoma or confined to the nodule in scirrhous carcinoma (6).

ACCURACY

Berger et al (1), concluded that in 10% of their cases the radiologist could not give an opinion, and that in those cases in which the radiologist could make a definite diagnosis of either malignant or benign, the accuracy was greater than 95% (i.e., less than 5% of malignancies were missed). Egan (3) examined 2000 cases and diagnosed 97% of the pathologically proven malignancies correctly. 9% of his benign cases were erroneously recorded as being malignant.

Whitten and Thurber (11) recently performed mammography on 5000 ASYMPTOMATIC women 40 years of age or older and revealed 8 preclinical carcinomas. They concluded from this that the routine use of mammography as a screening procedure for preclinical cancer could not be justified.

Accuracy is limited in the following situations: 7 (1) malignant masses located immediately under the nipple may appear benign; (2) masses located on the chest wall or in the periphery of the breast are difficult to detect; and (3) inflammations, abscesses, necrotic fatty tissue etc., may give the false appearance of malignancy.

RADIATION HAZARD (5)

In a single series involving three breast exposures, the average skin dose is 4r (range 2-10r depending on the size of the breast). This is greater than the skin dose of other routine radiological techniques such as chest film (6mr) or lateral exposure of pelvis and sacrum (3r); however, compared with the exposure permitted to radiation workers, it "does not seem excessive" (5). The doses expressed here indicate the amount of radiation received by the skin and not that received by the blood forming organs or the gonads (whole body radiation).

INDICATIONS FOR MAMMOGRAPHY (7, 8, 10)

1. Clinically questionable lesion in the breast;
2. Presence of multiple nodules in a patient who already received many previous biopsies;
3. Examination of fatty breasts;
4. Vague, indefinite breast complaints;
5. Anxiety or cancerphobia;
6. During pregnancy, when operation or anaesthetic is contraindicated (abdomen should be shielded);
7. Refusal of biopsy;
8. Examination of the opposite breast before radical mastectomy to rule out metastases or a second primary;

9. Follow-up examination of other breast at 6-12 month intervals following radical mastectomy;

10. Patients with metastatic disease in whom the location of primary is unknown.

In summary, it must be stressed again that mammography does not replace either physical examination or biopsy but rather serves as an aid when evaluated in the light of detailed history and physical examinations.

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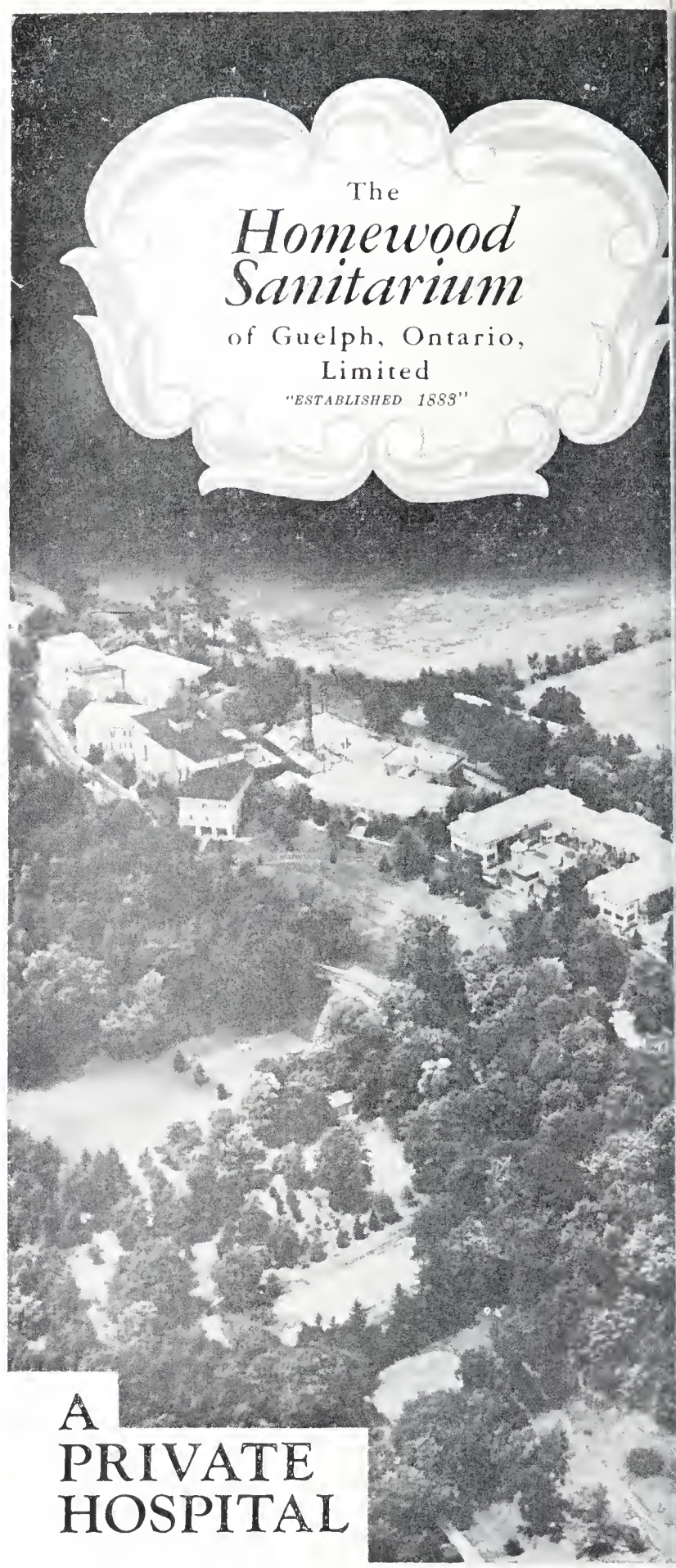

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Transformation of Lymphocytes *in Vitro**

M. A. Baker 6T6
D. H. Cowan, M.D., FRCP(C)†

In recent years much attention has been focused on the ole played by the lymphocytes which are found in the eripheral blood. A technique for studying peripheral lood leucocytes *in vitro* has been evolved in the last six ears which has yielded interesting and informative re-ults. This paper attempts a review of the recent *in vitro* xperiments and their implications concerning the function nd potentiality of the lymphocyte.

ULTURES WITH PHYTOHAEMAGGLUTININ

An important innovation in the technique of *in vitro* eripheral blood cultures was introduced by Hungerford al. in 1959 (1,2). In an experiment to study the cyto-enetics of peripheral blood leucocytes, a bean extract, hytohaemagglutinin (PHA), was added to whole blood amples to separate out the white cells in preparation for ulture. Subsequently, in cultures containing PHA, an in-rease in the number of mitoses of white cells was noted, fter three days of incubation. Virtually no mitosis oc-urred if PHA was not used. After ruling out all other ariables, Nowell (3) demonstrated that PHA was a spe-ific initiator of mitotic activity in human leucocytes.

Within the last six years several methods have been de-ised for including and observing the effects of PHA on hite blood cells. All these techniques involve the same eneral procedure. A heparinized sample of human venous lood is allowed to settle for one hour. The leucocyte-rich lasma superatant is then added with PHA to a suitable ulture medium. The cultures are incubated at 37°C. and re "harvested" after a minimum of 72 hours. Over the 2 hour period, some remarkable morphological changes ake place (4,5). These have been studied most commonly ith Jenner-Giemsa stain. Within the first 24 hours almost ll the neutrophilic granulocytes appear degenerated, and he few monocytes which were present seem to have dis-ppeared. The predominent cells at this stage resemble mall and medium lymphocytes. Over the next 48 hours, n increasing number of large primitive appearing cells re observed; so that at 72 hours, approximately 80% of he cells present are large, with abundant basophilic cyto-lasm containing distinct vacuoles. The nuclei of these ells are large and have distinct nucleoli, and a light stain-ng fine chromatin pattern. From 24 hours to 48 hours, nany cells are seen, which resemble transitional forms etween lymphocytes and the large blast-like cells. After 48 hours a gradual rise occurs in the number of mitotic figures seen.

When tritiated thymidine is added to a culture several hours before it is harvested, cells in the DNA synthesis phase of the cell cycle incorporate the radioactive DNA precursor, and can later be identified by autoradiography. This method of observation shows that cells which synthesize DNA increase in number within the 72 hour period from 0.5% to 45% (4). Both RNA and DNA synthesis have been shown to occur in sequence in the large blast-like mononuclear cells (6).

If the cultures are sustained for more than 3 days the primitive appearing cells will give rise to daughter cells resembling large, medium and small lymphocytes (5, 14). The *in vitro* experiments do not support suggestions by some, that mature lymphocytes undergo differentiation into red cells, granulocytes and histiocytes.

WHICH CELL IS TRANSFORMING?

The above observations seem to indicate that a "primitive" blast-like cell capable of division is being formed from some apparently "mature" cell circulating in the peripheral blood. It is of considerable interest to know which cell or group of cells is being transformed. It is consistently found that the majority of granulocytes degenerate and start to disappear within the first 24 hours of incubation (4). Since the blast-like mononuclears may not appear until 30 hours of incubation (5), the granulocytes may be eliminated as the source of the transforming cells (7, 2). Initially it was suggested that monocytes and relatively immature, large and medium sized lymphocytes were the only cells of those present in culture which were capable of division (1, 8). In cultures without PHA, monocytes and large and medium lymphocytes were the only cells to synthesize DNA (9). It was postulated that these same cells were also undergoing mitosis when PHA was added, but that PHA facilitated mitosis. The numerous small lymphocytes were regarded as having no potential for transformation or division.

Carstairs (10) observed, however, that the small lymphocytes began to disappear in cultures at the same time that the large blast-like cells began to appear. No significant decrease was noticed in the total number of cells present. MacKinney (4) et al. labelled the cells which were in DNA synthesis when drawn from the peripheral blood. He demonstrated that these cells divided at too slow a rate to account for the large number of mitoses subsequently seen when PHA was added. By studying the

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generation time of the dividing cells present in culture, it was suggested that the small lymphocyte was the only cell present in large enough numbers to be giving rise to the frequent mitoses (4, 11). Electron microscopic studies (14) have been consistent with the evidence that the proliferating large cells originate from small lymphocytes. Cytochemical studies demonstrate that the transformed large cells have staining properties which are characteristic of the lymphocytic series but not characteristic of the monocytic or granulocytic series (12).

Relatively pure suspensions of lymphocytes (88% to 100%) were obtained by using glass columns to separate the white cells of whole blood into fractions. Only the lymphocyte fraction could be induced to transform with PHA (13), and the same growth potential was exhibited by the lymphocyte fraction as was shown by whole blood (2). Further evidence that the small lymphocyte has the potential to transform is found in certain *in vivo* experiments (17, 18, 19).

These observations question the concept that the small lymphocyte is an end stage cell, unable to multiply (15, 10). Instead the evidence presented favours the concept that the small lymphocyte is a specialized cell in a relatively inactive state, but capable of transforming with appropriate stimulation (16, 4).

MECHANISM OF TRANSFORMATION

The mode of action of PHA is not entirely understood. While being used as a hemagglutinating agent, it was discovered to have a separate mitogenic property as well. The cells undergoing mitosis invariably clump together, and the suggestion has been made that PHA has some effect on the cell surface which induces clumping and provides a more favourable medium for growth (6, 20). A lapse of 24 to 48 hours always occurs before a significant number of mitoses are observed (3). This suggests that PHA does not stimulate the mitotic cycle *per se*, but initiates the conversion of susceptible cells to a state capable of division. Prednisone has been shown to inhibit this conversion if added during the first 24 hours of incubation (21).

Evidence suggests that the transformation caused by PHA may have an immunological basis (20). Labelled amino acid precursors added initially to cultures will appear in the protein of the supernatant. This labelled protein is in the gamma-globulin fraction electrophoretically, and all the lymphocytes in the culture combine with fluorescent anti-human gamma-globulin. The PHA molecules may be attaching to the cell surface and non-specifically stimulating the production of those gamma-globulins which the lymphocytes have been previously programmed to produce. On the other hand, fluorescent studies indicate that the gamma-globulin may represent specific antibody against PHA (22). Specific antibodies against the mitogenic properties of PHA have been produced in rabbit serum (23).

In these cultures the production of gamma-globulin always accompanied by transformation and mitosis of t small lymphocytes, but these cells have been shown transform and divide without producing gamma-glo ulin (20, 22).

A number of the known specific antigens such as C Tuberculin, smallpox vaccine and tetanus toxoid have be shown to produce transformation and mitosis of sm lymphocytes *in vitro* (20, 24, 22). This occurs only if t cell donor has been previously sensitized to the antig used as the *in vitro* stimulus. For example, if tetanus toxo is added to a cell culture from a donor previously sen tized, transformed and dividing cells are seen which a indistinguishable from those observed with PHA. As wi the PHA cultures the production of gamma-globulin c be demonstrated; however, in this case fluorescent tec niques show that the gamma-globulin represents speci antibody to tetanus toxoid. It is noteworthy that lymph cytic transformation is observed whether the antigen associated with delayed or immediate hypersensitivity *vivo*.

Cultures with PHA consistently yield 80 to 90% tran formation within three days. As a rule no more than 35 transformation over a period of seven days is obtained l stimulation with the specific antigens. It may be presum that only a limited number of lymphocytes are sensitize to a given antigen. It is unlikely, therefore, that PHA ac solely as a specific antigen causing a secondary immu ological response (24, 25). In fact, PHA causes the san degree of transformation and mitosis in fetal cells as it do in adult cells suggesting that prior sensitization is not a pr requisite (26). This primary response of lymphocytes PHA may be similar to the reaction of small lymphocyt to foreign antigens in graft-versus-host reactions and fir set homograft rejections (17). Attention is also drawn onc again to the possibility that PHA attaches to the surfac of lymphocytes and acts in some manner as a growt stimulating factor.

MIXED PERIPHERAL LEUCOCYTE CULTURES

The formation of large blast-like cells which underg mitosis can be caused by mixing peripheral blood leuc cytes from two separate donors (27, 28). In this case n other stimulant such as PHA is added to the cultures. Th small lymphocytes found in abundance in the peripher blood are thought once again to be the cells which tran form and divide. The resulting primitive appearing cel synthesize RNA and DNA and are identical in appearanc to the cells seen with PHA.

The adequate and necessary stimulus for transformatio is the addition of foreign leucocytes. Damaged, non-viabl leucocytes from one donor still produce transformatio in the other donor's living cells (29); however, foreig plasma, red cells, or platelets will not do so (27). Th basis of the mixed leucocyte reaction is thought to be th primary response of small lymphocytes to "individua specific" antigens present in leucocytes as well as in mo

ssues. The same antigens are said to be responsible for omograft rejections (28).

If the leucocytes of identical twins are cultured *in vitro*, o significant transformation is observed (27). If the subct pairs are fraternal twins or siblings, transformation is bserved, but to a lesser degree than seen with non-related onors. These findings suggest that genetic similarity etermines the degree of reaction observed.

A positive correlation was demonstrated between *in vivo* oss-reactivity studies and the degree of transformation mixed leucocyte reactions (29). A series of recipients ere sensitized by injections of leucocytes from donor A. kin was then transplanted to the same recipients from onor B. Rapid rejection of the skin grafts indicated ntigenic similarity between A and B, and correlated with low degree of transformation in their mixed leucocyte ltures. On the other hand, slow rejection of skin grafts orresponded to a high percentage of transformation in ltures.

These reactions observed in mixed leucocyte cultures ould form the basis of an *in vitro* histocompatibility test 27, 29, 30). It has been suggested that potential donorcipient pairs, being considered for renal homotransplantion, for instance, might have their immunological retionship evaluated by *in vitro* cultures. The lower the egree of transformation when their leucocytes are mixed, e better the chances of survival of the homograft (30). he peripheral blood is easily attainable for use as test aterial, and there is no danger of sensitizing the donor r the recipient to foreign antigens.

A further application of the *in vitro* leucocyte culture chnique has been suggested in relation to organ translants. The effectiveness of immunosuppressive therapy ay be evaluated by culturing the recipient's cells with HA. A high degree of transformation seen *in vitro* would ossibly indicate ineffective immunosuppression. A case as been reported(30) in which a rejection crisis was predicted several days before clinical manifestations of rejeon were seen.

ONCLUSIONS

The *in vitro* experiments suggest interesting possibilities bout the functions and potentialities of lymphocytes. It as now become widely accepted that small, apparently nature human lymphocytes are capable of growth and ransformation into large primitive appearing cells *in itro* (31). The latter cells have been shown to produce amma-globulin and undergo mitosis (20, 22). In certain ases the gamma-globulin has been demonstrated to repreent specific antibody. The exact mechanism by which HA produces transformation is as yet undetermined; owever, it is probable that the transformed lymphocytes ndergo primary immunological response. Transformation nd mitosis observed in the mixed leucocyte reaction furher support the hypothesis that prior immunization is not ecessary for lymphocyte transformation.

SUMMARY

A review of current literature is presented on the topic of *in vitro* leucocyte cultures. The evidence that the small lymphocyte undergoes transformation *in vitro* is summarized, and the various possible mechanisms of inducing transformation are discussed. Consideration is given to recent work concerning mixed leucocyte cultures.

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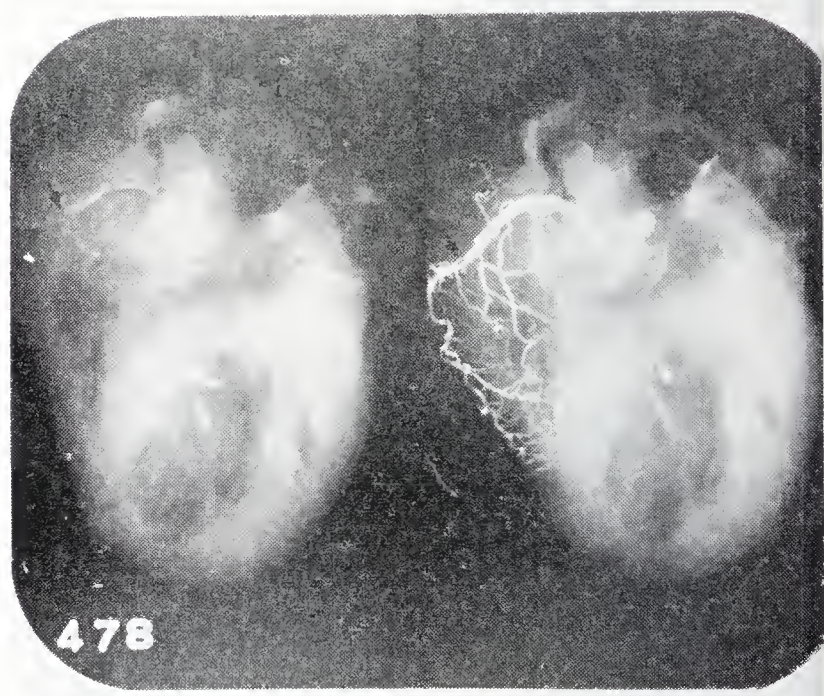
Tools of Research and Diagnosis

1. a) What diagnostic aid has been used?
   b) Diagnosis? Manifestations of this syndrome?
   c) What principle underlies the diagnostic method used?

3. The patient was admitted for investigation of a ur logical disorder, and on routine examination the abo X-ray was taken. Of what unrelated disease is this ray characteristic?

2. a) Of what diagnostic method is this record characteristic?
   b) Upon what principles does it operate?
   c) Indicate the conditions in which it might be appropriate to order this test.



4. Describe the X-rays and list the main causes of coror ary occlusion.

Answers on page 4

Ultrastructure and Current Concepts of Function of Mammalian Skeletal Muscle*

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INTRODUCTION

Understanding of function at the cellular and subcellular levels must include knowledge not only of the biochemistry involved, but also of the spatial relationships that functional units bear to one another. When such units are smaller than the resolving limit of the light microscope, the necessary morphological information must be supplied by the electron microscope. This is the case with skeletal muscle, in which the process of contraction involves the interaction of protein filaments smaller than 100 Å in diameter.

Although electron microscopy is far more adequate than light microscopy for morphologic investigation, it cannot at present routinely replace the latter because of its complexity and time requirement. The light microscope in its application to the detection and description of pathologic tissue will continue to be a useful and universally employed tool. But in such use it should always be borne in mind that a finer structure underlies all that is seen in the light microscope, and any understanding of what is seen must involve a knowledge of this finer structure. Such an approach to the interpretation of light microscopic findings will become even more important as the electron microscope is extended more broadly to the study of the mechanisms of pathology.

Thus, a light microscopic description can only serve as the beginning of an account of muscle structure as presently understood; the task of filling in the details falls to the electron microscope, as does the task of rendering visible the functioning units of muscular contraction.

LIGHT MICROSCOPIC STRUCTURE

Skeletal muscle appears in the light microscope as a parallel arrangement of long, slender muscle fibers bound together by delicate connective tissue.

A. *Muscle fiber*

Muscle fibers are highly elongated, cylindrical cells usually around 50 microns in diameter. Each displays a pattern of striations appearing as alternate light and dark stripes traversing the width of the fiber at right angles to its long axis.

*This work was supported by a Summer Fellowship and Grant from the Muscular Dystrophy Association of Canada.

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Sarcolemma:

The fiber is enshcathed by a thin membrane, the "sarcolemma", which appears after periodic acid Schiff staining, or when the fiber contents are artificially caused to retract. What is actually seen in the light microscope is probably a connective tissue component, to be described later as the basement membrane, and is not be confused with the true plasma membrane which is too thin to be resolved in the light microscope.

Nuclei:

An individual fiber possesses several nuclei, each elongated parallel to the long axis of the fiber and located at the extreme periphery of the cell. The nuclei each display several nucleoli.

Myofibrils:

The bulk of a fiber is seen on close examination to be almost completely composed of longitudinally disposed cylindrical components — myofibrils — tightly packed together and extending the length of the fiber. Alternate light and dark bandings on each myofibril are in register across the fiber and give the latter its uniform striated appearance. The darkly staining band is the A band, the lighter one is the I band. At high magnifications other bands, depicted in fig. 1—the M, H and Z bands—can also be discerned.

Sarcoplasm:

Traces of muscle fiber cytoplasm—sarcoplasm—appear within the fiber, and are especially evident in transverse sections, where they are sometimes to be seen separating myofibrils. When stained to advantage, granules of varying sizes appear in this sarcoplasm, some identified as glycogen, others as lipid, others as protein-lipid complexes called sarcosomes which are now known to be mitochondria.

B. *Connective tissue*

Individual muscle fibers are ensheathed and bound intimately to one another by a delicate connective tissue—the endomysium. This consists of fibrillar collagenous material and the occasional connective tissue cell. Blood capillaries and small nerve fibers are to be found coursing within the endomysium.

Fibers group together into bundles, or fascicles; these bundles, and groupings of such bundles, are separated by a more substantial connective tissue, the perimysium. This

provides support for larger blood vessels and nerves, as well as for lymphatic vessels. The entire musele is ensheathed in a dense connective tissue, the epimysium.

C. *Innervation*

Skeletal muscle receives motor innervation via axons whieh terminate on individual muscle fibers by entering into the formation of *motor end-plates*. The structural details of these are largely submieroscopie and will be mentioned later in this paper.

Sensory impulses, mainly proprioeeptive, originate from nerve endings on speeialized musele fibers. In plaees along their lengths these fibers are bound together by eonneetive tissue sheaths into fusiform bundles ealled *spindles*. The spindle fibers are eharaeterized outside the spindles only by their small diameters, but within the spindles also by a seareity of myofibrillar material, and by the aggregation of nuclei in the eenter of these fibers. The eleetron mieroseopic strueture of the highly specialized region (1) with its speeial innervation will not be described in this paper.

ELECTRON MICROSCOPIC STRUCTURE

A. *Muscle fiber*

Basement membrane:

In the eleetron mieroscope the musele fiber is seen to be surrounded by an extraeellular layer of a faintly granular amorphous material (BM in fig. 3) whieh is elosely applied to the eell membrane proper. This amorphous layer is eonsidered (2) to be the eounterpart in muscle of the "basement membranes" investing certain epithelial and endothelial eells of other tissues, and in eombination perhaps with some of the endomysial collagen is probably the membranous structure visible in the light mieroscope and denoted the "sareolemma" by the light mieroscopists. It merges outwardly with a network of collagen fibrils. Its composition, complex and thought to involve polysaeeharides (2), is largely unknown, as is its possible role as a differentially permeable membrane.

Cell membrane:

The plasma membrane appears in the eleetron mieroseope at moderate resolution as a dense line (PM in fig. 3) approximately 75 Å thiek. In eommon with the plasma membranes of all cells and with the membranes of a number of eell organelles, it has been shown (1) to possess a trilaminate or "unit membrane" structure, visible only at high levels of resolution. In this generalized eoneept of membrane strueture, the eell membrane is visualized as being eomposed of two protein monolayers, appearing in seetions as two dense lines, enelosing a lipid layer whieh appears as a region of lesser density.

The term "sareolemma" has been applied variously in the literature to the eell membrane, the basement membrane and the combination of the two. Its use has been avoided in this diseussion in favour of less ambiguous terms.

Periodically along its length the cell membrane shov small invaginations and numerous small intracellular v sieles just beneath it. These vesieles might be interprete as having just pinehed off from, or being just about fuse with, the eell membrane in the proeess of pinocytos described for other cells but not yet demonstrated musele.

Nuelei:

These elongated structures, lying peripherally in th musele fiber immediately beneath the plasma membran are bounded by a double membrane (two parallel men branes, eaeh displaying the unit membrane structure d seribed, and separated by a space of low electron density The interior of the nueleus displays an irregular stipplin of dense material whieh aggregates in plaees into dens elumps. Nueleoli appear as round dense bodies.

Golgi apparatus:

Frequently a Golgi apparatus ean be seen in the sarec plasm at one or both poles of a nueleus, taking the forr of a series of flattened membrane-bounded vesicles (3) These organelles do not seem to perform any conspicu ous funetion in the mature muscle eell, but appear to b more elaborately developed in regenerating muscle (4).

Mitoehondria:

The mitochondria of skeletal muscle, in common wit those of other cells, are roughly spherieal to elongate cylindrical bodies (fig. 6) composed of an outer membran of regular outline and an inner membrane which is throwi into folds, called cristae, extending inwardly into the mito chondrial matrix. The mitchondrial membranes and th matrix they enclose are the site of the enzymes of th Krebs citrie aeid cycle and of the process of oxidativ phosphorylation (redox ehain) which provide the eell wit its most important supply of energy-rich ATP.

Their distribution within the sarcoplasm of the muscl fiber is variable. They may appear singly or in aggregation at the poles of nuclei, anywhere immediately beneath th eell membrane, and singly or in long rows between myo fibrils where they are sometimes paired on opposite side of Z lines.

There are two, somewhat different, types of skeleta musele fiber which have traditionally been labelled as *red fibers* and *white fibers* on the basis of the gross appearanee of museles in which one or the other type predominates. The major reason for the different appearanee of these two types is the higher myoglobin content of the red fibers. Of perhaps greater *metabolic* signifieance is the considerably greater number of mitochondria possessed by the red fibers. Partly on this basis, with much supporting evidenee, it has been suggested (5) that in the red fibers, whose contraetion and relaxation times are slower, the citrie aeid eyele predominates as the means of energy utilization with fatty aeids and lipids as the main energy source, while in the white fibers, whose contraction times are more rapid, the glycolytie pathway predominates with

glycogen as the main energy source. The predominance of the citric acid-redox chain system with its capacity for high steady state ATP production renders the red fibers capable of sustained activity, while the predominance of the anaerobic glycolytic system makes the white fibers better suited to intermittent activity.

Glycogen granules:

After enhancement of preparation contrast with lead ions, glycogen appears in the form of granules of high electron density (6) in the sarcoplasm between myofibrils, at the poles of nuclei and just beneath the cell membrane (figs. 3, 4 and 7). Glycogen is more abundant in white fibers (5).

Ribonucleic protein (RNP) particles:

Our preliminary studies with preparations stained specifically for RNP indicate that these particles share a similar distribution within the fiber to that of the glycogen granules, lying freely in the sarcoplasm unattached to any membranes. In ordinary preparations they appear smaller and more regularly shaped than the glycogen granules (7), which largely obscure them. The basophilia of regenerating fibers observed in the light microscope is probably due to an increase in the number of these particles.

Myofibrils:

In the electron microscope, each myofibril appears as a parallel array of fine, longitudinally disposed myofilaments. The presence of both thin and thick filaments, and the arrangement of these with respect to each other, give rise to the banding pattern of the myofibrils. Figure 1 schematically depicts the fine structure of a relaxed myofibril. Two complete sarcomeres—portions of a myofibril between any two consecutive Z disks—are illustrated. (Compare fig. 1 with figs. 4 and 5.) Attention is directed to the highly ordered arrangement of thick and thin filaments as seen in cross sections of the myofibril at different levels, especially in the region where they interdigitate. By a number of different methods the identification of the thick filaments as myosin and of the thin filaments as actin has been achieved (8).

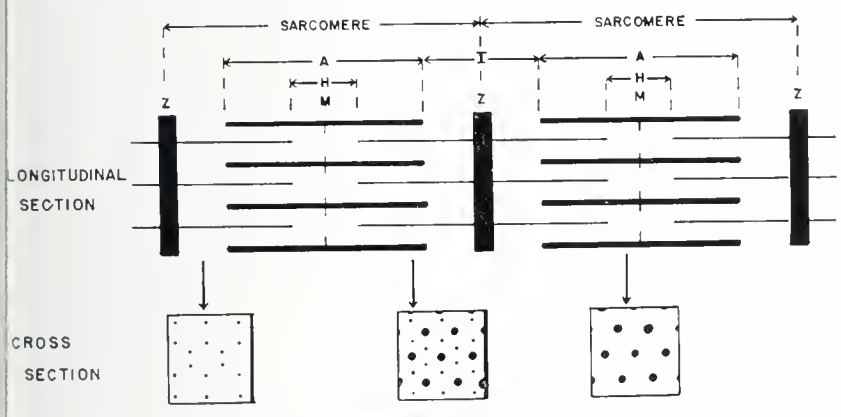

Figure 1. Diagrammatic representation of the structure of a single myofibril. The appearance on cross section varies according to the level of section.

At high levels of resolution the electron microscope reveals tiny lateral projections on the thick myosin filaments appearing on any one side approximately every 400 Å throughout the length of each filament. In the region where the actin filaments interdigitate with the myosin filaments, high resolution micrographs (9) seem to indicate that these projections from the myosin filaments attach to sites on the actin filaments, forming cross bridges. A 400 Å periodicity is also displayed by the actin filaments (10).

The actin filaments in each half sarcomere appear to terminate at either end of the sarcomere by attaching to the Z disks. The Z disk has been interpreted (11) as being made up of finer filaments which give attachment to the actin filaments and which are embedded in a material of moderate electron density.

Contractile function of the myofibril:

Convincing evidence exists to suggest that the structural basis of contraction is the drawing of the actin filaments into the A band. These slide between the myosin filaments, shortening each sarcomere by bringing the Z disks closer together. This "sliding filament" hypothesis (fig. 2) of contraction was first suggested on the basis of very careful light microscopic observations of the contraction of intact living fibers (12) and of isolated myofibrils (13). These revealed that in contraction over the physiological range the I and H bands shortened, while the length of the A band remained constant. The hypothesis received major support when the electron microscope subsequently established the interdigitating arrangement of the actin and myosin filaments (9). Although other, substantially different modes of contraction have been suggested, (14), in general, these are neither based upon, nor supported by, observations with *both* the light microscope (on living fibers) *and* with the electron microscope.

The tiny cross bridges between the myosin and actin filaments are widely regarded as being involved in the contractile process. It has been suggested (9) that in contraction the actin filaments may be drawn deeper into the A band by a cyclic process in which these side projections from the myosin filaments would attach to sites on the actin filaments, pull them a short distance toward the center of the sarcomere, release, and reattach further along the actin filaments to repeat the process. Evidence has been reported (8) which suggests that the myosin side projections possess ATPase activity when actin filaments are in close proximity, thus implicating these cross bridges in the energetics of contraction and giving support to such a model. A detailed molecular hypothesis based on this concept has recently been elaborated (15).

A variation of this model (16) envisages contraction by means of a repetitive deformation of the actin filaments. Attachment of the cross links to an actin filament along its length might stretch the actin filament, while a sequential releasing of the cross links starting from the top of the A band and moving toward the center of the band would cause the actin filament to "shrink" a short distance into the A band. In its new position new links could

form to stretch the actin filament again, and the process would repeat, resulting in further actin penetration into the A band.

An important question that has not yet been settled is whether or not part or all of the change in the sareomere length during contraction comes about as the result of the shortening of the actin filaments. It can be seen from fig. 1 that the actin filament length is the distance from the Z disk to the edge of the H band. If contraction is simply the sliding of the actin filaments between the myosin filaments without any shortening, then, in contraction, the decrease in length of the H band must equal the decrease in length of the sarcomere. However, the light mieroscopie studies on living fibres and isolated myofibrils already mentioned (12, 13) did not explicitly state that this was the case, while a recent study (17) has indieated that in contraction the small decrease in H band length observed in the electron microscope cannot account for the mueh larger decrease in sarcomere length. Accordingly, a "folding filament" model of contraction, shown sehematically in fig. 2, has been proposed (17) as a modifieation of the "sliding filament" model. The cross bridge meehanisms outlined could operate in the same way as proposed for the sliding filament model. However, as already stated, the question of filament length ehanges is by no means settled—changes in actin filament length during eontraction were *nont* found in another recent study (10).

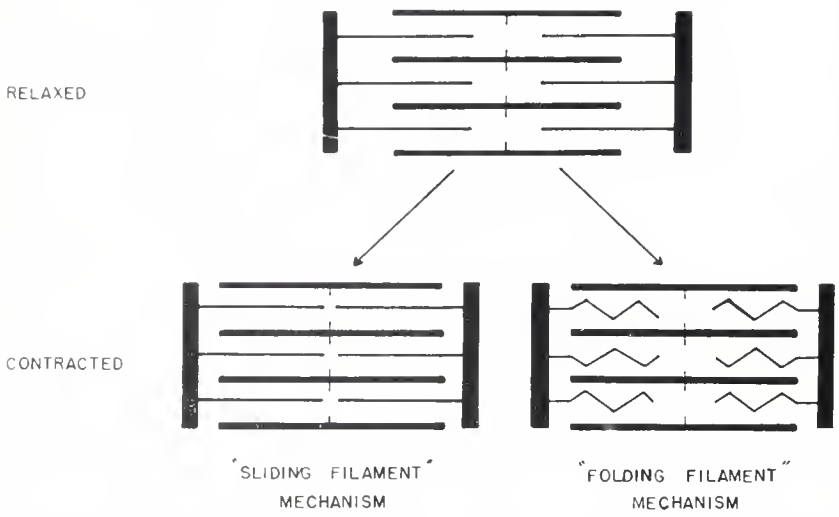

Figure 2. Two suggested modes of contraction are indicated diagrammatically.

Sarcoplasmic reticulum:

An anastomosing network of membrane-bounded tubules and cisternae, known as the sarcoplasmic reticulum, invests the myofibrils. It is composed of two morphologically distinct components, one disposed primarily longitudinally (LC in fig. 4), the other transversely; it is believed that there is no continuity between these components (3). The transverse portions consist of thin tubules pursuing courses over and around the myofibrils transversely across the fiber (TC in fig. 7) roughly at the levels, in mammalian muscle, where the A and I bands meet. The longitudinal portions consist of anastomosing tubules disposed between the myobrils. These become confluent to form cisternae eircling the myofibrils, in mammalian muscle, around the middle of each A band and the levels of the A-I junctions on either side of the transverse tubules. The arrangement in which two such cisternae sandwich a transverse element is referred to as *triad* (18, 6) (T in fig. 4).

Function of the sareoplasmie retieulum:

In part the sarcoplasmic reticulum seems to be the counterpart in muscle of the endoplasmic retieulum common to all cells, insofar as it is involved in the general metabolism of the cell. It has been reported to have a regulatory effect on the rate of glyeolysis in muscle (19) and to play an important role in glyeogen synthesis (20, 21). It is aetive in protein synthesis, although the RNP associated with it is not in partieulate form (22).

It is also believed that this counterpart of the endoplasmic retieulum in musele represents a form highly specialized to play a number of roles in the process of contraction. Firstly, it is a system (relaxing faetor system) (23) and/or is the souree of a substanee (relaxing faetor) (24) which by inhibiting the myofibrillar ATPase is capable of causing the relaxation of muscle fibres in the presenee of ATP. (In the absence of this faetor ATP induces contraction.) Secondly, it is reported to have a great eapacity to bind the concentrate Ca^{++} (25), a significant eapability in the light of the susceptibility of the relaxing faetor to inhibition by Ca^{++}. The Ca^{++} binding eapaeity may be localized in the plasma membrane and the transverse tubules (6). Thirdly, the sarcoplasmie retieulum has been widely eonsidered to be the means whereby the contractile impulse, or action potential, is eonducted into the depths of the fiber from the eell membrane. (An action potential is a propagated ehange in the electrical potential normally present aeross the eell membrane, usually induced by arrival at the myoneural junetion of a nerve impulse.)

This latter role has been suggested by a number of observations. The morphologieal eounterpart of the transverse tubules in *cardiac* muscle is a series of invaginations of the cell membrane deep into the cardiae fiber (26). Although this has not been demonstrated to be the case in skeletal muscle, the transverse portions (as well as the longitudinal portions) do appear to eome into very close apposition to the cell membrane. The most suggestive evidence for this role has been the demonstration (2) that the areas of the cell membrane of skeletal musele which are most responsive to electrical stimulation (i.e., where contraetion can be induced by the weakest stimulus) are those areas where the triads of the sarcoplasmic reticulum lie just beneath the cell membrane. Lastly, the presenee of ATPase aetivity in the membranes of the sarcoplasmic reticulum (23, 27) makes *possible* the conduction of an action potential by the reticulum. ATPase aetivity in the membrane is generally associated with aetive transport of Na^+ and K^+ and their differential concentration on opposite sides of the membrane. It is this differential eoncentration that is, in part, responsible for the electrie properties of membranes.

Connective tissue

Bundles of parallel collagen fibrils and the occasional broblast make up the connective tissue between muscle bers. The fibroblast cytoplasm, drawn out into long, ender processes, usually displays an abundance of rough rfaced reticulum appearing as membranes studded with ectron-dense RNP particles and enclosing tubular lumens. broblasts characteristically lack a basement membrane.

Capillaries

The cytoplasm of the endothelial cells forming the blood pillaries contains small vesicles suggestive of a pinocyto- exchange between the lumen of the capillary and the tracellular space. The outer plasma membrane of the dothelial cell is covered with a basement membrane.

Motor end-plate

A myelinated motor axon branches repeatedly in its urse within a muscle, resulting finally in a number of yelinated terminal axons, each destined to terminate on different muscle fiber. Close to the fiber which it innertes, a terminal axon loses its myelin sheath and imediately divides into several branches which come to lie ose together in depressions—*synaptic gutters*—in a single ised mound of muscle sarcoplasm containing numerous uclei and mitochondria — the *soleplasm*. The muscle asma membrane is thrown into a series of infoldings in is region. Schwann cells bridge the gutters over the axon anches, and the whole arrangement so formed is the otor end-plate. The release of acetylcholine occurs here n arrival of a nerve impulse, triggering on action potenal along the muscle cell membrane, but the exact site om which the acetylcholine is released has not been tablished (28).

ONCLUSION

The mechanisms of muscular contraction are not yet nown with any certainty. However, on the basis of ochemical and morphologic investigations a consensus as evolved, and the following sequence of possible events representative of current speculation (25, 6, 15, 29).

) A wave of depolarization sweeps along the cell membrane of a motor axon to reach the axon endings at a motor end-plate. The arrival of the impulse triggers the release of acetylcholine from somewhere in the region of the end-plate.

) The release of sufficient acetylcholine causes a depolarization of the muscle cell membrane in the end-plate region. An action potential spreads along the muscle plasma membrane away from the motor end-plate.

) Wherever a triad is located beneath the cell membrane, the action potential is conducted into the depths of the fiber along the membranes of the sarcoplasmic reticulum. In this way the action potential reaches the region of each individual sarcomere.

4) As the action potential sweeps along the reticulum, it triggers the release of Ca^{++} from the reticulum into the region of the myofibrils, where it acts to inhibit the relaxing factor. The inhibition of the relaxing factor restores to activity the ATPase residing in the cross bridges (29), and these in turn become involved in the process by which the actin filaments are pulled into the A band region. A prerequisite for contraction to occur is the presence of ATP, produced from energy-releasing processes occurring in the mitochondria (citric acid cycle), at the sarcoplasm-sarcoplasmic reticulum interface (glycolysis), and in the sarcoplasm (other systems).

5) The sarcoplasmic reticulum takes up the Ca^{++} previously released, and possibly also releases relaxing factor. The Ca^{++} uptake restores to activity the relaxing factor, which in turn inhibits the cross bridge ATPase allowing relaxation to occur.

Besides that of contraction, there are other problems of function pertaining to muscle, and, indeed, to all cells, especially concerning the mechanisms of cell maintenance and repair and aberrations in these essential processes. It is in these areas in particular that the task of correlating ultrastructure with function is just beginning.

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FIGURES

3. Adjacent portions of two fibers sectioned longitudinal run from top to bottom. Parallel myofibrils (MF) lie clo together with their bandings in register to give the cros striated appearance to the muscle fiber. In the fiber the right Golgi vesicles (G) and a nucleus (N) are visibl The inset is an enlarged portion of the fiber on the le and in it the basement membrane (BM) and plasma men brane (PM) can be seen. 25,000

4. This micrograph shows one complete sarcomere in long tudinal section. Compare the filamentous structure wit the diagram in fig. 1. Four triads (T) and a tubule of th longitudinal component (LC) of the sarcoplasmi recticulum are evident. Note the aggregations glycogen granules (Gl). 68,000

5. This cross section of a portion of one fiber shows myo fibrils (MF) outlined by the sarcoplasmic reticulum. Th imperfect register of the myofibril bandings in this fibe gives varying cross sectional appearances from one myo fibril to another. The sarcomere levels (Z, I, A, H) ar labelled. Compare with fig. 1. 73,000

6. In this longitudinal section of a portion of a fiber, larg elongated mitochondria (Mit) are conspicuous betwee myofibrils (MF). Note the long finger-like cristae extend ing into the mitochondrial matrix. 25,000

7. The plane of section includes the superficial myofilament of 4 sarcomeres and the overlying sarcoplasmic reticulum The transverse tubules (TC) of the latter are readil apparent. Glycogen granules (Gl) are also conspicuous 107,000

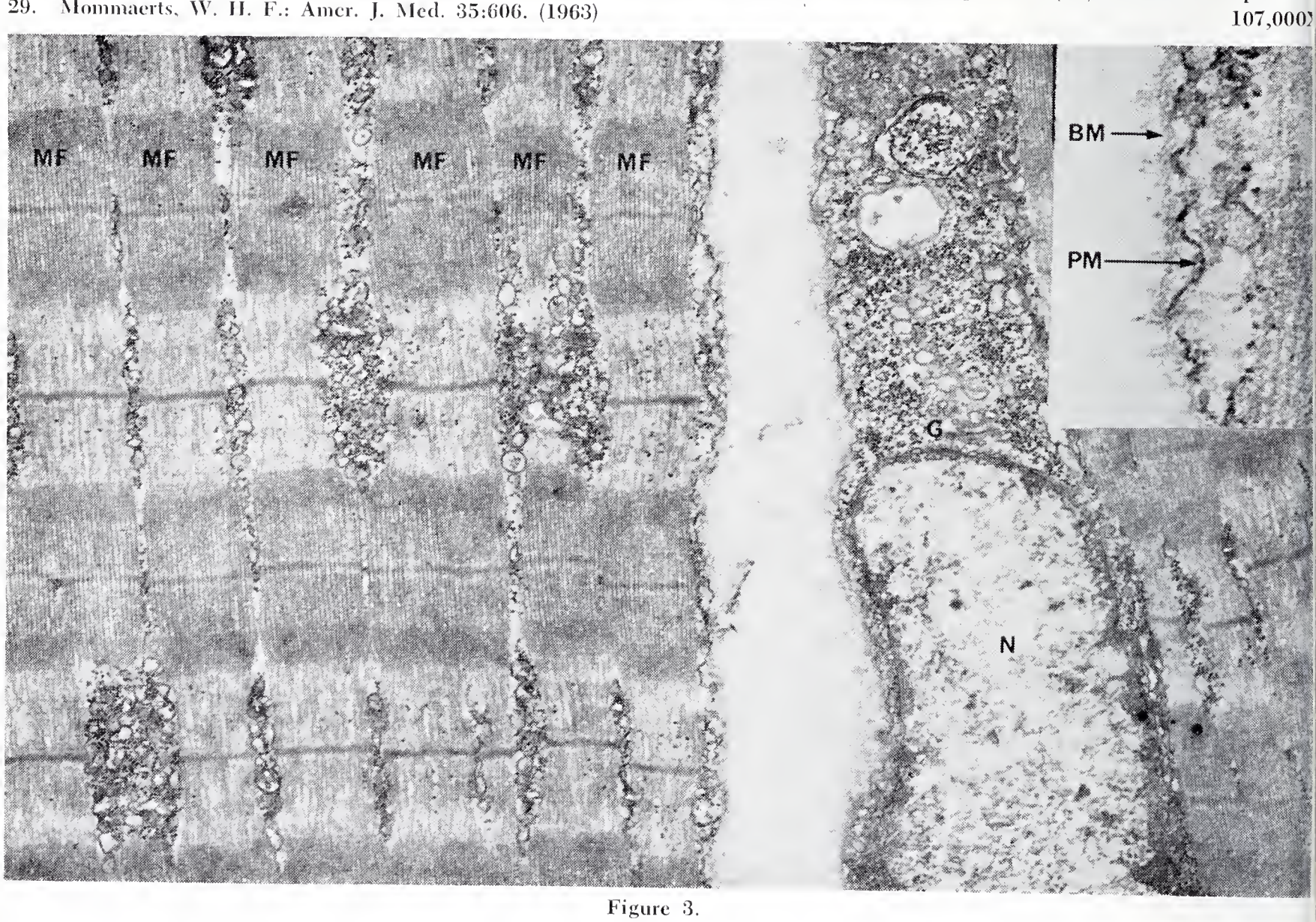

Figure 3.

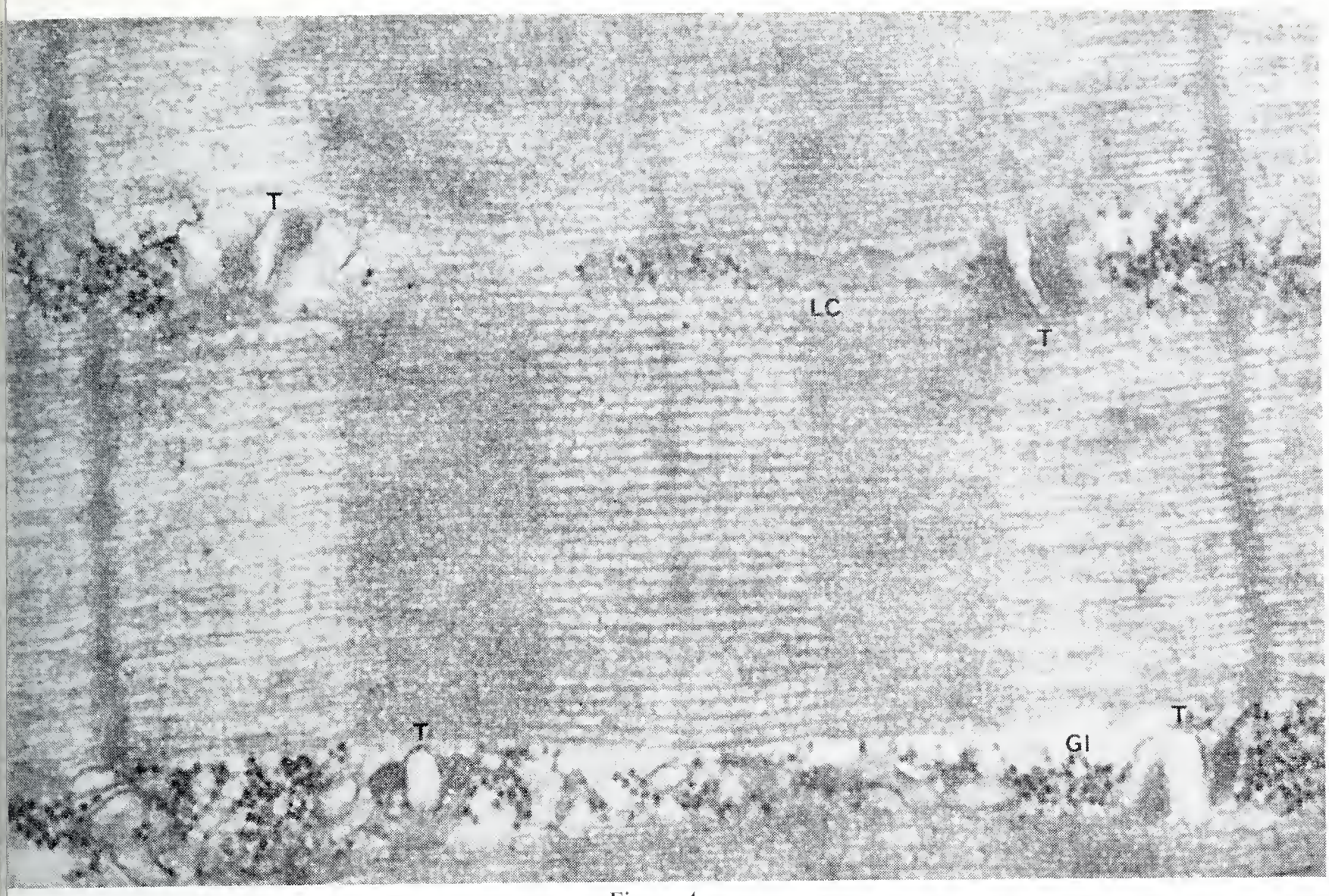

Figure 4.

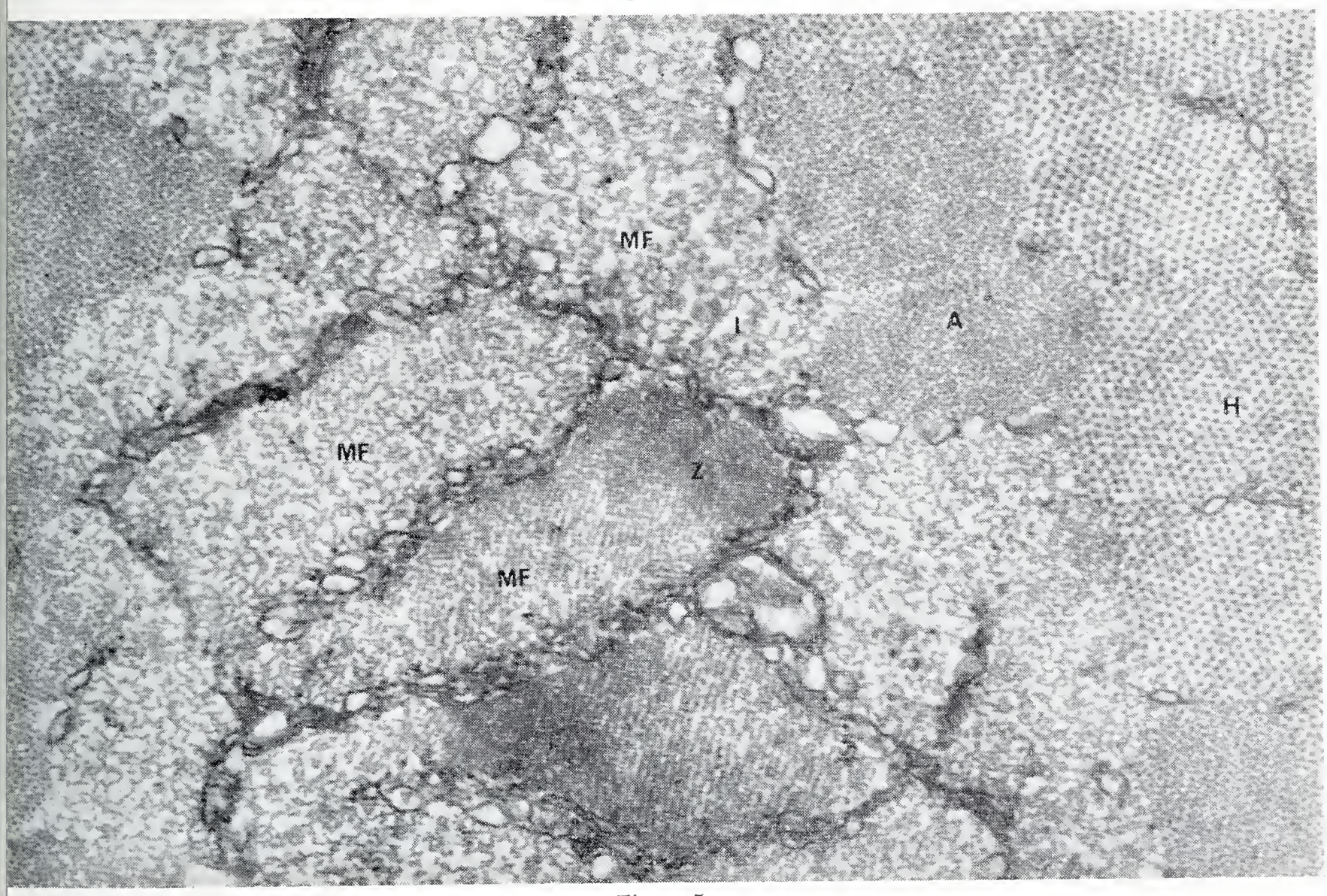

Figure 5.

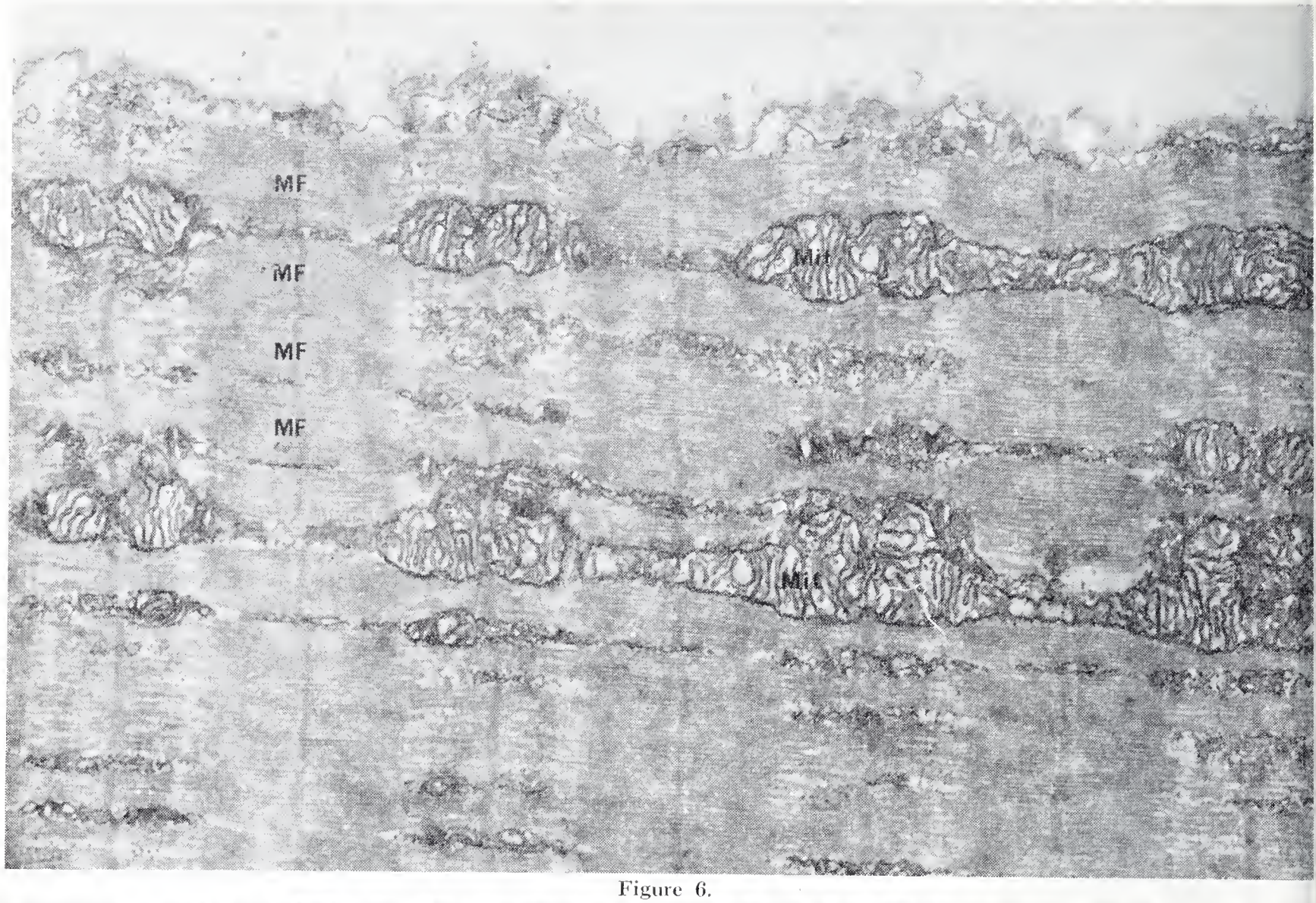

Figure 6.

Figure 7.

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On Inspection - Answers

. a) A normal and an *infrared photograph* of the same atient are compared. In the lower infrared photograph, large number of distended vessels are seen on the upper hest.

b) This patient has a primary carcinoma of the bronhus, which has produced the *superior vena caval syndrome*. The symptoms of this syndrome are produced by a) occlusion of the superior vena cava and (b) the involvement of other mediastinal structures.

Occlusion of the superior vena cava results in:

1) Oedema of face, neck, conjunctivae, vocal cords (which may produce voice changes);
2) Distension of veins in the neck, chest and arms (note this patient's distended external jugular veins);
3) Development of collateral circulations (note the "girdle of Venus");
4) Severe headaches, epistaxis, syncope, changes in vision.

Invasion of other mediastinal structures may give rise o such manifestations as paralysis of recurrent laryngeal erve, hiccoughs, paradoxical diaphragm.

c) Infrared photography, by means of specially sensiive film, records superficial changes in skin temperture; e.g., distended blood vessels. A more recent advance n this general field is infrared thermography, which meaures deeper changes in temperature such as occur over tumour mass and inflammation. This latter method also ecords "cold spots"; e.g., cysts, dead tissue, fat. The neasurements for thermography are done by a "heat amera" and a scanning device. The clinical uses of both nfrared photography and thermography are extending into he fields of peripheral vascular disease, vascular obstrucion, active rheumatoid arthritis, and possibly the screenng of carcinoma of the breast.

2. a) The record presented is typical of the liver scan btained from the *Radioactive Rose Bengal Test*. The three eparate black markers are the positions of the right and eft lower costal margins and centrally the umbilicus. From the scan, the liver notch, and the right and left lobes of the liver may be discerned. The upper border of the iver has been drawn in.

b) The liver parenchymal cells have a purifying action upon certain substances, particularly dyes bound to serum albumin. The hepatic parenchyma clearance can be measured by sulfobromophthalein (B.S.P.), uranin (fluorescein), and rose bengal dyes. The Kupffer cells do not participate in the taking up and excretion of these dyes.

In the case of rose bengal, the dye is injected intravenously, captured by the liver, and rapidly excreted in the bile. If one labels rose bengal with I^{131}, one can measure variations in radioactivity (as a function of time) by a scintillation counter over the hepatic area. The scan obtained has been presented. Interpretation of the test depends upon variations from the normal rate of uptake and excretion of radioactive rose bengal by the liver. For example, damaged or altered parenchymal cells produce a smaller and/or slower rise in radioactivity; if the biliary tract is blocked the curve reaches its maximum level in the same time but excretion is delayed.

c) From this relatively simple test it is possible to:

1) Locate primary and secondary malignant lesions;
2) Locate cysts, vascular disturbances and malformations, etc.;
3) Assess liver parenchymal function (e.g., cirrhosis);
4) Diagnose obstructive biliary lesions.

Rose Bengal Scan has become an important and popular aid to diagnosis.

3. The X-ray shows many ovoid areas of soft-tissue calcification. This X-ray appearance is diagnostic of *cysticercosis*. Although rarely seen in this country, the X-ray appearance is striking and will alert one to the presence of this disease in an asymptomatic patient.

The disease is caused by the pork tapeworm, Taenia solium. In its usual clinical form, man obtains the disease by eating undercooked pork containing the cysticerci, which then allows the scolex to become free and attach itself to the intestinal mucosa and the adult worm to develop. Occasionally man will ingest the ova, or a gravid segment will return to the stomach by reverse peristalsis. The released embryo burrows into the intestinal wall and is distributed by vascular channels to various parts of the body to form cysticerci, particularly in striated muscle, and of greater significance, in the eye and brain. During the stage of actual larval encystment, symptoms of muscular pain, weakness and a slight fever may occur. Involvement of the brain may produce encephalitis or a form of epilepsy. As the cysticerci eventually degenerate calcification occurs and presents the X-ray appearance illustrated. Eosinophilia is an aid to diagnosis. There is no treatment, although surgery may be indicated in cerebral and ocular cysticercosis. Always keep this disease in mind as a rare cause of epilepsy since it may be curable.

4. The first film demonstrates *massive coronary calcifications* involving all 3 major vessels; i.e., right coronary, as well as left anterior descending and circumflex branches of the left coronary artery.

Injection of a radio opaque material into the right coronary artery demonstrates a complete block (arrow), which in this case was associated with a myocardial infarction. All the vessels are markedly narrowed but show anastomotic channels past the site of the block. Occlusion of the right coronary is usually associated with a left ventricular infarct.

The main causes of coronary occlusion include:

1) Atheromatous plaques: usually complicated by haemorrhage into the plaque, or rupture of the plaque, or thrombosis over the clot;

2) Syphilitic aortitis and occlusion of the coronary ostia;

3) Embolism: either vegetative from the aortic valve, fat from a fracture, nitrogen in Caisson's disease, etc.;

4) Dissecting aneurysm of the arch of the aorta and occlusion of the coronary ostia;

5) Associated with polyarteritis nodosa;

6) Associated with hypercoagulability states such as polycythemia or sickle cell anemia.

Note: Significant calcification is found in over 80% of people over 60 years of age. It appears later in females (possibly due to the effect of the female sex hormones). The importance of accessory coronary ostia as a source of anastomotic channels has not been sufficiently stressed. In a recent series by Dr. E. L. Lansdown of the Department of Radiology of the Toronto General Hospital, 58% of postmortem hearts had more than 2 coronary ostia, while as many as 5 separate ostia have been found in a single specimen.

The assistance of Mr. Fred Simon, Dr. E. L. Lansdowne and Dr. F. W. Simpson in the preparation of "On Inspection" is gratefully acknowledged.

Abstract

"Dear Doctor, Please See"
Reflections on the Work of a General Pediatric Outpatient Clinic

Macaulay, Duncan: Lancet, 2:400-403, Aug. 22 (196

860 Patients were seen at a pediatric outpatient clin 90% referred by family doctors. The problems encou ered were, therefore, representative of those confronti general practitioners.

In all age groups, 10% of cases had no abnormalit and reassurance of parents and doctor prompted the cc sultation. Organic disorders decreased from 80% in t first year to 45% in the 9-15 year-old group. Non-orgar disorders, on the other hand, increased from 5% in t first year to 50% in the 9-15 year-olds. Many of the were common behavioral problems.

Because of the multitude of non-structural diseases a because many doctors feel uneasy when confronted wi them, the author strongly advises better training of medic students in the recognition and treatment of these comm disabilities, this training probably best being obtained a medium-sized, provincial, non-teaching hospital. In d scribing a plan of future outpatient departments, he su gests consideration for those needing "time and priva rather than access to machinery".

M.

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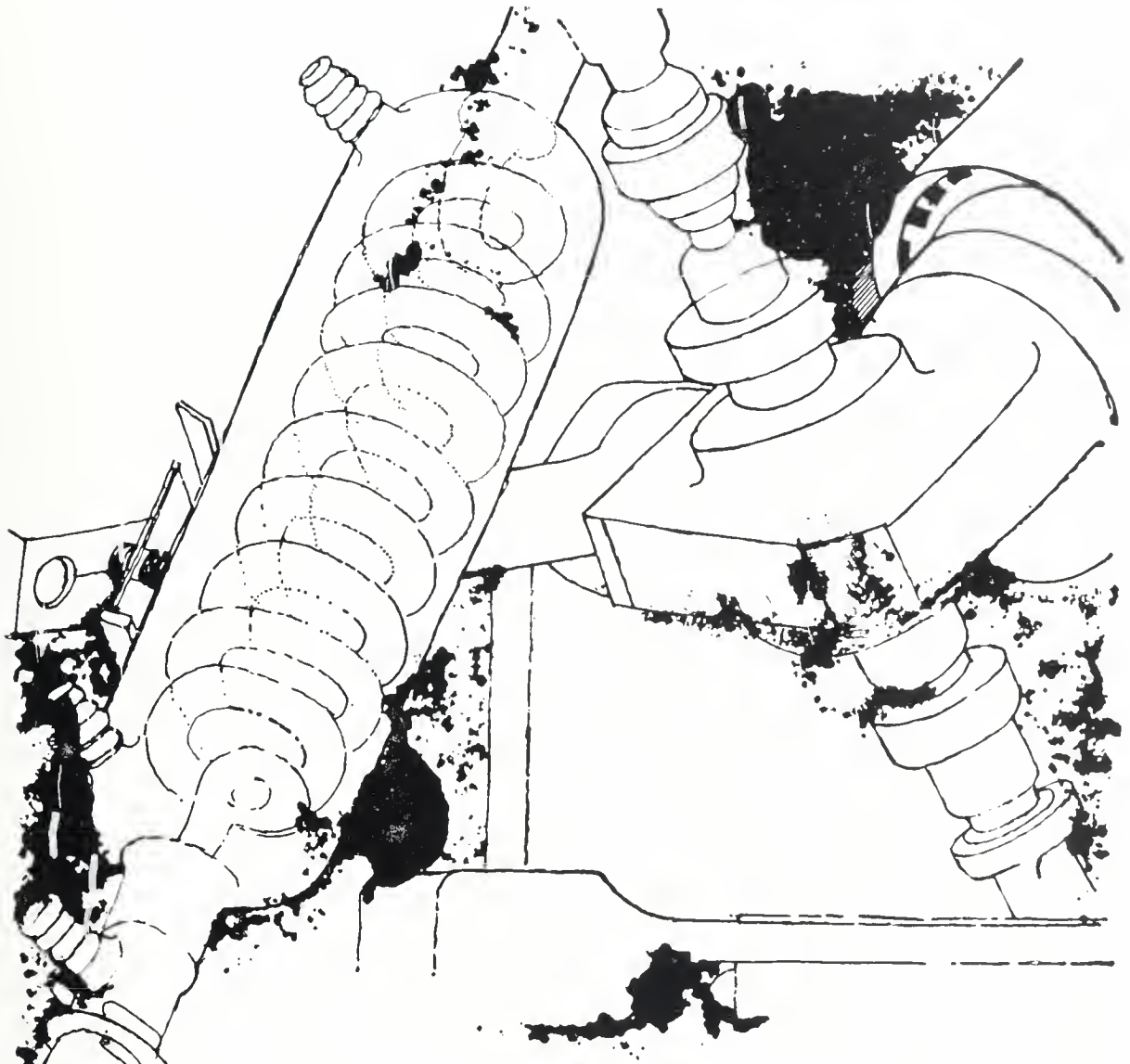

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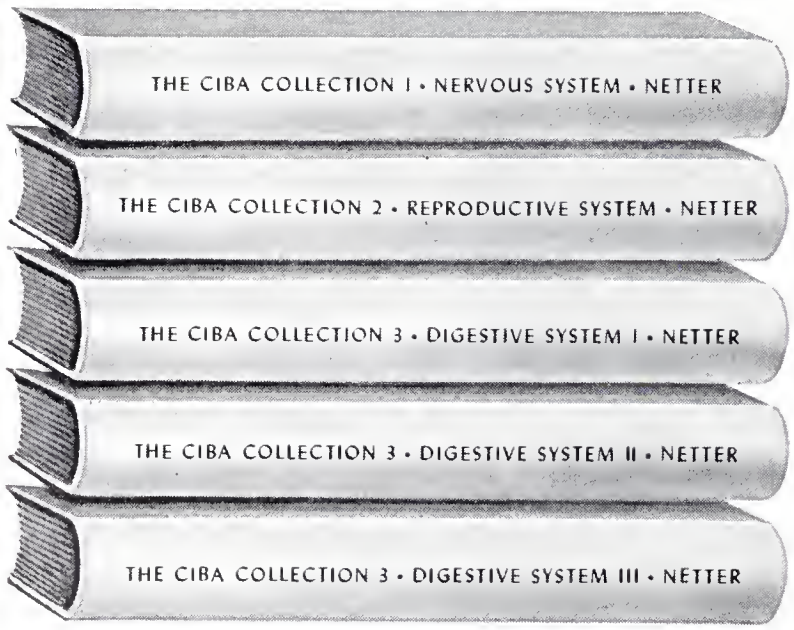
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